

Immunohematology and Transfusion Medicine

A Case Study Approach

Second Edition

Mark T. Friedman
Kamille A. West
Peyman Bizargity
Kyle Annen
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Springer

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Mark T. Friedman
Icahn School of Medicine
Mount Sinai Health System
New York, NY
USA

Kamille A. West
Department of Transfusion Medicine
National Institutes of Health Clinical Center
Bethesda, MD
USA

Peyman Bizargity
Department of Molecular & Human
Genetics
Baylor College of Medicine
Houston, TX
USA

Kyle Annen
Department of Pathology
Children's Hospital Colorado
Aurora, CO
USA

Jeffrey S. Jhang
Icahn School of Medicine
Mount Sinai Health System
New York, NY
USA

ISBN 978-3-319-90959-2 ISBN 978-3-319-90960-8 (eBook)
<https://doi.org/10.1007/978-3-319-90960-8>

Library of Congress Control Number: 2018946662

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The authors proudly dedicate this workbook to those who have tirelessly invested their professional careers and lives working to advance the science, knowledge, and practice of blood banking, immunohematology, and transfusion medicine: to those who pioneered; to those who continue to discover and build on the foundations; and to those who will inherit the wisdom to become the innovators and educators of tomorrow...

Preface

Pretransfusion testing, including ABO/Rh typing, identification of unexpected antibodies, and compatibility testing, is an important measure in the provision of blood that may be transfused to the patient in the safest possible manner. This brief introduction is not intended to give the trainee a detailed instruction on solving immunohematology cases; rather, it is intended to give an overview on how to approach the immunohematology problems (Chaps. 1–36) of this workbook. The authors of this workbook presume that the reader has had at least basic instruction in immunohematology before engaging in these cases.

Although one may be tempted to jump right to the antibody panel after noting a positive antibody screen in the presented cases, it is recommended to review the clinical history for important clues that may be helpful in solving the case. For example, a history of prior transfusions suggests that the patient could have made clinically significant alloantibodies (i.e., warm-reactive immunoglobulin [Ig]G alloantibodies capable of causing hemolytic transfusion reactions or hemolytic disease of the fetus or newborn). Alternatively, the use of phrases such as “routine clinic visit” may suggest that the patient is clinically stable despite significant anemia. In these practice cases, as in the real medical world, obtaining clinical history is an important step not to be overlooked, though in some of these cases (as sometimes occurring in actual practice), scant history is provided.

After reviewing the medical history, the next step is to interpret the ABO/Rh typing results. In most cases, this will be straightforward, though one should be alert to any discrepancy in the forward and reverse typing results. For example, noting a positive result with the A₁ cell in the back type may be the result of anti-A₁ antibody in an individual of A₂ blood type or the result of a cold allo- or autoantibody.

Next, one should review the antibody screen. It should be noted that in this workbook, we present a two-cell screen in either standard tube or gel (column agglutination) method. Although typically the antibody screen is interpreted simply as positive or negative, limited additional information can be gleaned by noting differences in reactions between the two cells (i.e., whether both cells or only one cell reacting) or differences in the testing phases (i.e., if tube method is used, differences in reactions between 37 °C vs. antihuman globulin [AHG] phase). Additionally, the

antigen profiles of the antibody screen cells are listed in the beginning, which may also provide useful information when ruling out antibodies.

After review of the clinical history, ABO/Rh typing, and antibody screen, one is ready to move on to the antibody panels if performed in the case (see Fig. 1). Although traditionally one is taught to interpret the antibody panels through a process of crossing out antigens, it is prudent to first take a moment to get a “landscape” view of the panel reactions. That is, one should look to see whether there are reactions at cold temperatures (i.e., 4 °C, room temperature [RT], immediate spin [IS]) or warm temperatures (37 °C, IgG), whether there are many cells that are positive (perhaps all cells are positive as in a panagglutinin reaction) or only few, and whether the autocontrol is positive or negative. Such consideration may help to narrow the possible specificities of the present antibodies. In that light, for example, if reactions are only evident at 4 °C in the panel, then warm-reactive antibodies (such as anti-D, -K, -Jk^a) can promptly be excluded. Finally, after this initial review, one should then move on to the methodical exclusion of antibody specificities. This is traditionally taught as “crossing out” antigens in which the reactions are negative with attention toward dosage (i.e., homozygous vs. heterozygous antigen expression). Figure 2 demonstrates crossing out with respect to negative reactions, dosage, and the patient’s RBC antigen phenotype. The effect of enzyme treatment (e.g., papain or ficin) may also be of value as antibody reactivity to some antigens may be enhanced or destroyed. Ultimately, after consideration of all of the clinical information and antibody identification testing, the identity of the antibody or antibodies may be determined so that the most compatible blood can be provided for the patient in case transfusion is necessary.

In the end, these cases are not necessarily meant to be difficult (though they do become more challenging as one progresses through the workbook) but are selected based on principle to introduce the practical concepts of and methods used in immunohematology antibody identification. Once the learner has grasped these basic techniques, he/she can apply them to more interesting cases that may be presented to them within the actual clinical practice of the transfusion service.

Finally, Chaps. 37–56 are designed to engage the learner in other aspects encompassing transfusion medicine including coagulation, hematopoietic stem cell transplantation, granulocyte transfusion, massive transfusion, therapeutic apheresis, factor concentrates, transfusion complications, patient blood management, and the approach to the bloodless patient.

New York, NY, USA
Bethesda, MD, USA
Houston, TX, USA
Aurora, CO, USA
New York, NY, USA

Mark T. Friedman
Kamille A. West
Peyman Bizargity
Kyle Annen
Jeffrey S. Jhang

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Fig. 1 Guide to the antibody panel

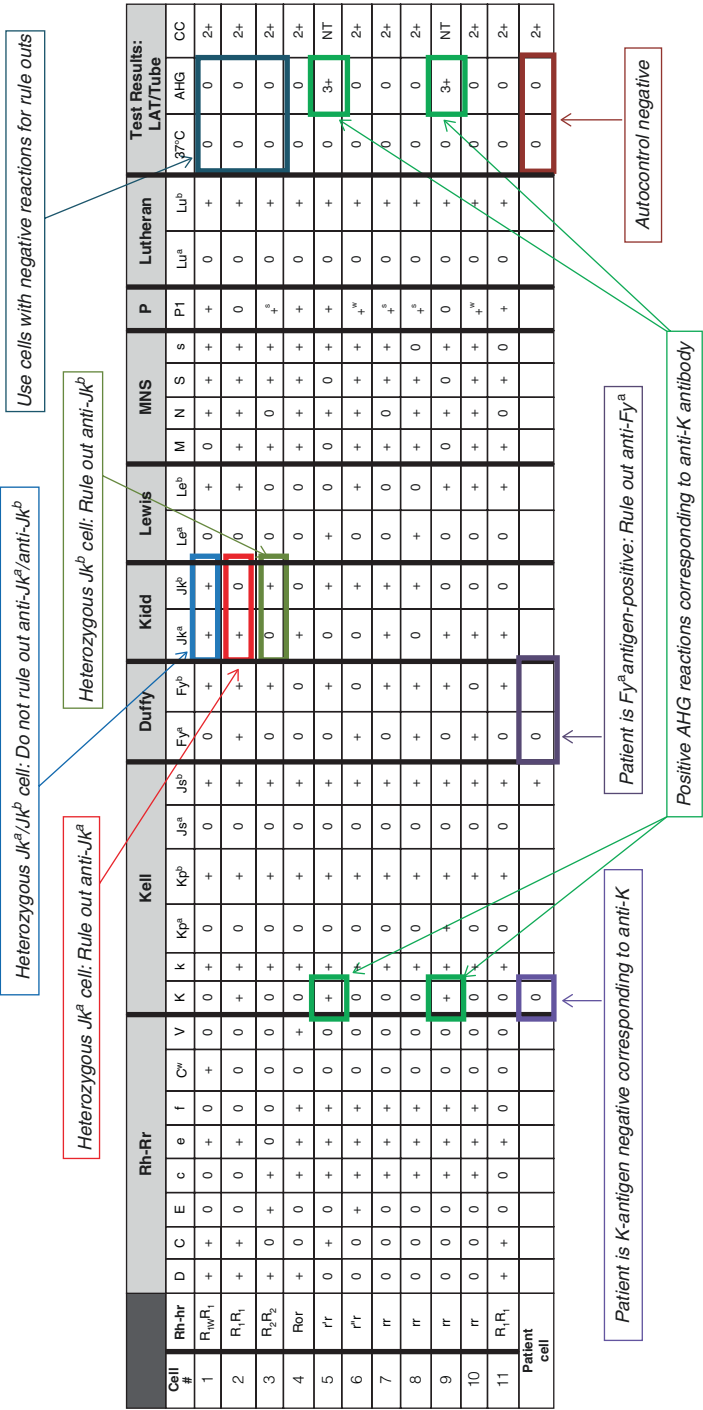


Fig. 2 Guide to antibody panel rule out

Authors' Note

This case-based immunohematology workbook, now in its second edition with added cases and enhanced information and references, has been developed over many years and was created in response to the need to teach learners in the practical art of interpreting red cell antibody studies. Though there have been many textbook resources, both in print and more recently online, which have been published for learning facts about blood banking and transfusion medicine, there are few available resources for studying and practicing immunohematology cases. The cases presented in this workbook are based on realistic clinical scenarios that one may encounter in the blood bank laboratory and transfusion service. Thus, this workbook is an excellent companion to reference texts for the practical application of learned immunohematology techniques to case-based studies in the identification of red cell antibodies and in the clinical management of patient transfusions. Educators will find this workbook to be a valuable resource among their collection of teaching tools. The questions accompanying each case are presented in an open-ended format rather than in the traditional single-best-answer multiple-choice format; though some may prefer the latter format for convenience, it is wise to remember that patients do not come with multiple-choice answers. Furthermore, the open-ended style should hopefully encourage learners to engage in further reading and discussion of the subject matter. Over the years, many trainees in clinical pathology and hematology have benefitted from the use of this type of workbook problem-solving learning, both in the preparation for their board certification exam and for their careers, and since the publication of the first edition, the workbook has achieved international acclaim.

The authors are indebted to Sandra Gilmore, Sr. Director of Patient Blood Management/Bloodless Care in the Mount Sinai Health System, New York, NY, for her expertise on the approach to the “bloodless patient” and patient blood management in the preparation of this workbook.

For questions or comments, please send e-mail to Dr. Mark Friedman at mark.friedman@mountsinai.org or follow us on our **facebook** page.

Kyle Annen
Peyman Bizargity
Mark T. Friedman
Jeffrey S. Jhang
Kamille A. West

Table of laboratory normal values

Laboratory test		Normal range
White blood cells (WBC)		3.8–9.8 K/ μ L
Hemoglobin (Hgb)		12.5–17.0 g/dL
Hematocrit (Hct)		34–46%
Mean corpuscular volume (MCV)		80–100 fL
Platelets		150–450 K/ μ L (150,000–450,000/ μ L)
Reticulocyte count		0.5–1.5%
Haptoglobin		30–200 mg/dL
Lactate dehydrogenase (LDH)		300–600 U/L
Total bilirubin		0.2–1.3 mg/dL
Creatinine		0.6–1.3 mg/dL
Prothrombin time (PT)		11.0–14.0 s
International normalized ratio (INR)		1.0–1.2
Activated partial thromboplastin time (aPTT)		32.0–40.0 s
Urinalysis	Color/Turbidity	Yellow/Clear
	Red blood cells (RBCs)	0–3/High power field
	Urine hemoglobin	Negative
	Urine myoglobin	Negative

Table of RBC antigen frequencies

Blood group system	Antigen	Frequency (%)		
		Caucasian	African American	Asian
Rh	D	85	92	99
	C	68	27	93
	c	80	96	47
	E	29	22	39
	e	98	98	96
Kell	K	9	2	–
	k	>99	>99	–
Duffy	Fy ^a	66	10	99
	Fy ^b	83	23	18.5
Kidd	Jk ^a	77	92	73
	Jk ^b	74	49	76
MNS	M	78	74	–
	N	72	75	–
	S	55	31	–
	s	89	93	–

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List of Abbreviations

ACT	Activated clotting time
ADAMTS13	A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
ADSOL	Additive solution
AFIA	Arrayed fluorescence immunoassay
AHG	Antihuman globulin
AIHA	Autoimmune hemolytic anemia
aHUS	Atypical hemolytic uremic syndrome
ALI	Acute lung injury
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
aPTT	Activated partial thromboplastin time
ARDS	Acute respiratory distress syndrome
ASFA	American Society for Apheresis
AST	Aspartate aminotransferase
BNP	Brain natriuretic peptide
BPDR	Biological Product Deviation Report
CABG	Coronary artery bypass graft
C ₃ b, C ₃ d, C ₄ b	Complement components 3b, 3d, 4b
CBER	Center for Biologics Evaluation and Research
CC	Coombs control/Check cells
CCI	Corrected count increment
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CGD	Chronic granulomatous disease
CHF	Congestive heart failure
CLL	Chronic lymphocytic leukemia
CMV	Cytomegalovirus
CPB	Cardiopulmonary bypass
CPDA	Citrate–phosphate–dextrose–adenine
CPOE	Computer physician order entry

CS	Cold storage
C-section	Cesarean section
CT	Computed tomography
DAT	Direct antiglobulin test
D-D	D-dimer
DDAVP	Desmopressin acetate (arginine vasopressin)
DIC	Disseminated intravascular coagulation
DIHA	Drug-induced hemolytic anemia
D-L	Donath–Landsteiner
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphates
DOB	Date of birth
DRVVT	Dilute Russell viper venom test
DTI	Direct thrombin inhibitor
DTT	Dithiothreitol
D5W	5% dextrose in water
D10W	10% dextrose in water
ECG	Electrocardiogram
ECMO	Extracorporeal membrane oxygenation
ED	Emergency department
EDTA	Ethylenediaminetetraacetic acid
EGA	EDTA glycine acid
ELISA	Enzyme-linked immunosorbent assay
F	Factor
FDA	Food and Drug Administration (United States)
FDP	Fibrin degradation products
FFP	Fresh frozen plasma
FMH	Fetal-maternal hemorrhage
FNAIT	Fetal/neonatal alloimmune thrombocytopenia
FNHTR	Febrile nonhemolytic transfusion reaction
G-CSF	Granulocyte colony-stimulating factor
GERD	Gastroesophageal reflux disease
GI	Gastrointestinal
G#P#	Gravida #, Para #
GPA, GPB	Glycophorin A, B
G6PD	Glucose-6-phosphate dehydrogenase
GVHD	Graft-versus-host-disease
HBOC	Hemoglobin-based oxygen carrier
HBV	Hepatitis B virus
Hct	Hematocrit
HCV	Hepatitis C virus
HDFN	Hemolytic disease of the fetus/newborn
HEMPAS	Hereditary erythroblastic multinuclearity with positive acidified serum test
Hgb	Hemoglobin
HIT	Heparin-induced thrombocytopenia

HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HLA-PRA	Human leukocyte antigen-panel reactive antibody
HNA	Human neutrophil antigen
HPA	Human platelet antigen
HSC	Hematopoietic stem cells
HSCT	Hematopoietic stem cell transplant
HTLA	High titer/low avidity
HUS	Hemolytic uremic syndrome
IAT	Indirect antiglobulin test
ICH	Intracranial hemorrhage
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
IND	Investigational new drug
INR	International normalized ratio
IS	Immediate spin
ISI	International sensitivity index
ITP	Idiopathic (immune) thrombocytopenic purpura
IU	International units
IV	Intravenous
IVIG	Intravenous immunoglobulin
K	Kinetic time (thromboelastography)
KB	Kleihauer–Betke
LDH	Lactate dehydrogenase
LISS	Low ionic strength solution
LMWH	Low molecular weight heparin
LY30	Lysis (clot lysis at 30 min—thromboelastography)
m	Microscopic
MA	Maximum amplitude (thromboelastography)
MACE	Modified antigen-capture enzyme-linked immunosorbent assay
MAIPA	Monoclonal antibody immobilization of platelet antigens
MCV	Mean corpuscular volume
mf	Mixed field
MICU	Medical intensive care unit
MPS	Massively parallel sequencing
MRN	Medical record number
MSC	Marrow stem cells
NAT	Nucleic acid test
NHSN	National Healthcare Safety Network
NGS	Next-generation sequencing
NOACS	Novel anticoagulants
NT	Not tested
PAS	Platelet additive solution
PBM	Patient blood management

PBSC	Peripheral blood stem cells (PBSC)
PCC	Prothrombin complex concentrate
PCH	Paroxysmal cold hemoglobinuria
PCR (AS)	Polymerase chain reaction (allele-specific)
PEG	Polyethylene glycol
PF4	Platelet factor 4
PFC	Perfluorocarbon
pHUS	Pneumococcal-associated hemolytic uremic syndrome
PI	Pathogen inactivation
PICU	Pediatric intensive care unit
PLS	Passenger lymphocyte syndrome
PRCA	Pure red cell aplasia
PT	Prothrombin time
PTP	Posttransfusion purpura
R	Reaction time (thromboelastography)
RBC	Red blood cell
RCo	Ristocetin cofactor
RESt	Rabbit erythrocyte stroma
RhAG	Rh-associated glycoprotein
RhIg	Rh immune globulin
RT	Room temperature
S	Strong
SC1	Screen cell 1
SC2	Screen cell 2
SCD	Sickle cell disease
Se	Secretor
SNP	Single nucleotide polymorphism
SPRCA	Solid-phase red cell adherence
STEC	Shiga toxin <i>Escherichia coli</i>
SRA	Serotonin release assay
TACO	Transfusion-associated circulatory overload
TA-GVHD	Transfusion-associated graft-versus-host disease
TJC	The Joint Commission
tPA	Tissue plasminogen activator
TPE	Therapeutic plasma exchange
TRALI	Transfusion-related acute lung injury
Tryp	Trypsin
TTP	Thrombotic thrombocytopenic purpura
UCBSC	Umbilical cord blood stem cells
UFH	Unfractionated heparin
VWD/F	von Willebrand disease/factor
W	Weak
WAIHA	Warm autoimmune hemolytic anemia
WBC	White blood cell
WBIT	Wrong blood in tube

Chapter 1

Basic Single Antibody Identification: How Hard Can It Be?



Clinical History

A 52-year-old man with a history of hypertension, type 2 diabetes mellitus, and three-vessel coronary artery disease is admitted to the hospital for a coronary artery bypass graft (CABG) surgery. An ethylenediaminetetraacetic acid (EDTA) anticoagulant sample is submitted to the blood bank for type and screen along with an order for four units of red blood cells (RBCs). No transfusion history is given.

ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	3+	4+	4+
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	0	0	2+	
SC2	3+	4+	NT	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Tube Panel

Cell #	Rh-hr	Rh-hr							Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube LISS					
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P _I	Lu ^a	Lu ^b	37 °C	AHG
1	R _{1w} R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	0	0	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	+	0	0	0	0	0	2+
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	+	+	+	0	3+	4+	NT
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	0	0	0	2+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	0	+	+	+	0	0	0	2+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	3+	4+	NT
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	0	0	0	2+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	+	0	0	0	2+
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	0	0	+	0	+	+	+	0	0	0	0	2+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	+	0	0	0	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	+	+	+	+	0	0	0	2+
Patient cell																												0	0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What is the patient's ABO/Rh blood type?
2. What antibodies did you identify?
3. Are the antibodies clinically significant? Why or why not?
4. How many RBC units would you need to screen in order to find four compatible (i.e., negative for the corresponding antigen) units as requested? (Refer to the Table of RBC Antigen Frequencies, using antigen frequencies listed under Caucasian population)

Answers

1. **What is the patient's ABO/Rh blood type?** The patient is group O, Rh-positive blood type. Forward or front typing of the patient's sample (i.e., using reagent anti-A and anti-B sera to detect A and B antigens on the RBCs) shows that the patient is group O type (i.e., neither A nor B antigens are detected). Back or reverse typing of the sample confirms that patient is group O since both anti-A and anti-B isoantibodies are detected in the plasma of the patient. Testing with reagent anti-D serum shows that the Rh(D) antigen is present on the patient's RBCs; therefore, the patient is Rh(D)-positive.
2. **What antibodies did you identify?** Anti-E alloantibody is present; Rh(E) antigen (Rh3) is a part of the Rh blood group system. Although the "rule of three" applies in the identification of antibodies, for simplicity of working up the cases in this workbook, the learner will find that the rule cannot be consistently applied. The "rule of three" states that at least three antigen-positive and three antigen-negative RBCs that react and do not react, respectively, are necessary to achieve a statistically significant p value (or probability value) of 0.05 to rule-in or rule-out an antibody. This standard approach, based on Fisher's exact method, is necessary to minimize the risk of a false-negative (i.e., failure to identify an alloantibody that is present) or a false-positive (identification of an alloantibody that is not present) result [1]. In this case, however, only two of the panel cells are Rh(E)-antigen positive, and so a third cell technically should be tested; in actual practice, blood banks utilize secondary antibody panel kits for such additional testing as needed.
3. **Are the antibodies clinically significant? Why or why not?** Anti-E is a clinically significant alloantibody since it is immunoglobulin (Ig)G, is warm temperature reactive (i.e., 37 °C), and is capable of causing delayed hemolytic transfusion reactions as well as hemolytic disease of the fetus/newborn. In general, clinically significant antibodies are warm-reacting, immune IgG antibodies, while cold-reacting IgM antibodies are generally not considered to be clinically significant. Antibodies to the following blood group antigens are usually IgG:

Rh, Kell, Duffy, Kidd, and Ss. Antibodies to the following blood group antigens are usually IgM: Lewis, MN, and P1.

4. **How many RBC units would you need to screen in order to find four compatible (i.e., negative for the corresponding antigen) units as requested?**

Since approximately 29% of the Caucasian population carries the Rh(E) antigen (see Table of RBC Antigen Frequencies) on their red cells, 71% do not carry it. The chances of finding a compatible donor red cell unit for the patient with an anti-E antibody are about seven out of ten units. A total of four RBC units were requested for the patient. Dividing 4 by 0.71 (i.e., $4/0.71$), we find that 5.6 or, essentially, six RBC units need to be screened in order to find four Rh(E)-antigen-negative, compatible RBC units for the patient. However, one must also keep in mind that because the patient's blood type is O-positive, as noted above in question 1, only group O units may be screened for this patient. In terms of prevalence, group O is 45% in the Caucasian population (while group A is 40%, group B is 11%, and group AB is 4%).

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Chapter 2

Rhesus Pieces



Clinical History

A 25-year-old woman, gravida 2, para 1 (G2P1), at 37 weeks of pregnancy, who has not had any complications throughout the pregnancy, presents for a routine prenatal clinic visit. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

ABO/Rh (tube method)				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4 +	0	0	0	4 +
Antibody screen (tube LISS method)				
	37 °C	AHG	CC	
SC1	1 +	2 +	NT	
SC2	1 +	2 +	NT	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Tube Panel

Rh-hr														Kell				Duffy		Kidd	Lewis		MNS			P	Lutheran		Test results:				
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lur ^a	Lur ^b	37 °C	AHG	CC		
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	+	1+	2+	NT
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	0	+	+	1+	2+	NT
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	+	+	0	+	+	1+	2+	NT
4	R ₀ R	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	+	0	+	+	1+	2+	NT
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	0	+	+	0	0	2+
6	r'r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	+	+	+	0	+	+	0	0	2+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	0	+	+	0	+	+	+	0	+	+	0	0	2+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	+	+	+	0	+	+	0	0	2+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	2+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+	0	+	+	0	0	2+
11	R ₀ r'	+	+	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	+	+	+	0	+	+	0	W+	NT
Patient cell																														0	0	0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What is the patient's ABO/Rh blood type?
2. What antibodies did you identify?
3. Can the weaker reaction strength on cell #11 seen in the panel be explained?
4. What are the possible causes or sources of the antibodies in this patient?
5. What additional testing would you do in the blood bank to help you determine the nature or source of the antibodies?

Answers

1. **What is the patient's ABO/Rh blood type?** The patient is group A, Rh-negative blood type. Refer to Chap. 1, question 1 answer for further information on forward and reverse ABO typing.
2. **What antibodies did you identify?** Alloantibody against the Rh(D) antigen (Rh1) is present.
3. **Can the weaker reaction strength on cell #11 seen in the panel be explained?** Cell #11 is an R_0r' (Dce/Ce) red cell, meaning that the Rh(D) antigen is in the *trans* position (i.e., on the opposite allele) relative to the Rh(C) antigen. Rh(D) antigen expression is weakened by the steric arrangement of the Rh(C) antigen (known as the "Ceppellini" effect) [1]. Thus, the panel shows weaker reactions with these cells from the anti-D antibody present in the patient's serum. It is best to use R_2R_2 red cells when testing for weak- or low-titer anti-D antibodies. However, in actual practice, this effect is not commonly seen on panels but is illustrated here as a teaching point. See the table below for a review of the Rh haplotypes [2]; an easy way to remember this table is to know that $R = D$, $r = d$, $0 = ce$ (R_0 or r), 1 or $' = Ce$ (R_1 or r'), and 2 or $" = cE$ (R_2 or r''). It is also helpful to remember that R_0 , R_1 , R_2 , and r are the common four haplotypes.

Wiener haplotype	Fisher–Race haplotype	Wiener haplotype	Fisher–Race haplotype
R_1	DCE	r	ce
R_2	DcE	r'	Ce
R_0	Dce	r''	cE
R_z	DCE	r'	CE

4. **What are the possible causes or sources of the antibodies in this patient?** We are not given a transfusion history in this pregnant patient, but it is possible that she was transfused with Rh(D)-positive blood products in the past, either mistakenly or in the case of an emergency when sufficient Rh-negative blood was not available. It is also possible that the patient developed anti-D antibodies as a result of fetal–maternal hemorrhage, either during the current pregnancy or during prior pregnancies (including abortion or fetal loss). Given that the current

pregnancy is uncomplicated, the most likely explanation for the presence of anti-D antibodies is passive administration of anti-D (i.e., Rh immune globulin, RhIg). RhIg (300 μ g dose) is routinely given at 28 weeks of gestation to Rh-negative women who have not been previously sensitized [2].

5. **What additional testing would you do in the blood bank to help you determine the nature or source of the antibodies?** Besides careful history taking, including all pregnancies, abortions, transfusions, and RhIg injections, the titer of anti-D antibodies could be helpful in distinguishing anti-D from active immunization (i.e., exposure to Rh-positive red blood cells) versus passive immunization (i.e., RhIg). A low titer of anti-D (i.e., titer ≤ 4) would favor passively acquired anti-D versus higher titers of the antibody. A room-temperature indirect antiglobulin test (IAT) may also be of value since the presence of such reactions would indicate the presence of immunoglobulin (Ig)M anti-D (i.e., newly developing anti-D); IgM is not present in manufacturer RhIg preparations and thus would indicate active immunization. In any case, a history of RhIg injection should always be elicited to confirm the suspicion of passively-acquired anti-D [2].

References

1. Ceppellini R, Dunn LC, Turri M. An interaction between alleles at the Rh locus in man which weakens the reactivity of the Rh0 factor (D0). *Proc Natl Acad Sci U S A*. 1955;41:283.
2. Denomme GA, Westhoff CM. The Rh system. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff CM, editors. *Technical manual*. 18th ed. Bethesda: AABB; 2014. p. 320.

Chapter 3

Crossmatch Crisscross



Clinical History

A 41-year-old woman with Crohn's disease presents for outpatient transfusion. The patient has a history of symptomatic chronic anemia as per the patient's gastroenterologist and has received several blood transfusions at another hospital in the past, though none in the past 2 years. An ethylenediaminetetraacetic acid (EDTA) anticoagulant sample is submitted to the blood bank (properly labeled with the patient's last name, first name, medical record number [MRN], and date of birth [DOB]: "SMITH, JULIE; MRN 1001234; DOB 3/20/####*"; * denotes year 41 years ago from current date) for type and screen along with a request for crossmatch of two red blood cell (RBC) units. There are no prior test (i.e., type and screen) results in your blood bank information system; in fact, this is the first admission to your hospital facility for this patient (i.e., a new patient).

ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	3+	0	4+
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	0	0	2+	
SC2	0	0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

The patient receives the two units of RBCs in the outpatient transfusion suite without incident and is discharged home. The patient returns to the hospital for outpatient transfusion 8 months later (second admission), and another EDTA sample is received in the blood bank, labeled “WILLIAMS, JULIE; MRN 1001234; DOB 3/20/####.” There is a request for one unit of RBCs. Test results are as below:

ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	3+	0	4+
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	0	0	2+	
SC2	0	0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Further History

Upon inquiry by the blood bank supervisor regarding the patient’s last name, the nurse in the outpatient transfusion department relates that the patient got married in the interim after the initial visit. The nurse emphatically verifies that it is indeed the same patient. The patient is transfused a unit of RBCs without incident and is discharged home. One year later, the patient is admitted to the hospital (third admission) for surgery, and a preoperative EDTA sample is received in the blood bank for type and screen; the results are below:

ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	3+	0	4+
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	0	0	2+	
SC2	3+	4+	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Tube Panel

		Rh-hr							Kell				Duffy		Kidd	Lewis		MNS				P	Lutheran	Test results: IAT/tube LISS								
		D	C	E	c	e	f	c ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	37 °C	AHG	CC	
Cell #	Rh-hr																															
1	R ₀ wR ₁	+	+	0	0	+	0	0	+	0	0	+	0	+	+	0	+	+	+	0	+	0	+	+	+	+	+	+	0	0	0	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	0	0	+	0	0	+	+	+	0	0	+	0	0	0	2+
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	0	+	0	+	0	0	+	0	0	0	0	0	+	0	+	+	+	+	3+	4+	NT	NT
4	R ₀ r	+	0	0	+	+	+	0	+	0	0	+	0	+	0	0	0	+	0	0	0	0	+	+	+	+	+	0	0	0	0	2+
5	rr	0	+	0	+	+	+	0	0	0	0	+	0	+	0	0	+	0	+	+	0	0	0	+	+	+	+	0	0	0	0	2+
6	r ⁺ r	0	0	+	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	0	0	+	+	+	+	+	+	+	3+	4+	NT	NT
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	0	0	+	+	+	+	+	0	0	0	0	2+
8	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	+	+	+	0	+	+	0	0	+	+	+	0	0	0	0	0	0	2+
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	0	+	0	+	0	0	+	0	+	0	0	0	0	0	0	0	2+
10	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	0	0	+	+	+	+	+	+	0	0	0	0	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	0	+	0	0	0	+	+	+	+	+	+	0	0	0	0	2+
Patient cell																												0	0	0	0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What is the patient's ABO/Rh blood type?
2. What method would you use to perform compatibility testing (i.e., crossmatching) for this patient based on the sample and test results of the first admission, and what would be the ABO/Rh type of the RBCs that you would crossmatch? What risk is involved?
3. What method would you use to perform compatibility testing for this patient based on the sample and test results of the second admission, and what would be the ABO/Rh type of the RBCs that you would crossmatch? What risk is involved?
4. What method would you use to perform compatibility testing for this patient based on the sample and test results of the third admission?

Answers

1. **What is the patient's ABO/Rh blood type?** The patient's blood type is group A, Rh-positive. Refer to Chap. 1, question 1 answer for further information on forward and reverse ABO typing.
2. **What method would you use to perform compatibility testing (i.e., crossmatching) for this patient based on the sample and test results of the first admission, and what would be the ABO/Rh type of the RBCs that you would crossmatch? What risk is involved?** There are three options for performing compatibility testing (also known as crossmatching) in the United States: immediate spin (IS) crossmatch, electronic (computer) crossmatch, and antihuman globulin (AHG, full) crossmatch. The type of crossmatch performed depends on whether the patient has any clinically significant antibodies, either currently or by history, the number of type and screen samples tested in your facility, and whether the blood bank has a validated information (computer) system capable of performing compatibility testing by electronic crossmatch. In this case, during the first admission for the patient, immediate spin crossmatch could be performed for the two requested RBC units, given that the patient has only one sample tested for ABO/Rh type and that the antibody screen is negative while the patient has no known history of clinically significant antibodies. The advantage of IS crossmatch is that it is a serologic crossmatch that can be performed within a short time (a few minutes), enhancing turnaround time for blood issue. The main disadvantage is that IS crossmatch is only capable of detecting ABO compatibility; thus, the patient could suffer from a delayed hemolytic reaction if an undetectable alloantibody was to be present in the patient. However, this risk is considered to be acceptably low enough such that IS crossmatches may safely be performed in the majority of patients (one exception may be sickle cell disease [SCD] patients in which 20–50% may develop clinically significant alloantibodies so that some blood bank facilities make it a policy to use the AHG crossmatch

for all SCD patients regardless of history and the current antibody screen result [1]. Alternatively, an AHG crossmatch (also known as a full crossmatch) could be performed, capable of testing for compatibility with ABO antibodies as well as antibodies to other RBC antigens if present. The disadvantage of the AHG crossmatch is that it requires up to 1 h to perform, thus impacting turnaround time and work flow in the blood bank laboratory. Meanwhile, the electronic crossmatch technically may be performed for this patient at this time, since the patient's single sample may be retyped (electronic crossmatch requires two ABO determinations, which may be done by retyping the same sample but is preferably done by typing of two different samples, though one of the samples may be historic [i.e., comparison to a previous typing on record], see question 3 answer below for full details on the electronic crossmatch). Although one may wish to crossmatch type-specific (i.e., A-positive) RBCs for this patient based on the single type and screen sample tested in consideration of good inventory management (i.e., minimizing overuse of group O blood), the risk of doing so is in the event of patient misidentification, commonly referred to as "wrong blood in tube" (WBIT). In WBIT, the sample is properly labeled with the correct patient information (full name, MRN, DOB) but is collected from the wrong patient. This typically occurs when the sample tube is labeled at the nursing station (or other location distant from the patient's bedside) and the patient's identification is not confirmed at the time of sample collection. To prevent this potentially devastating error (since it may result in transfusion of ABO-incompatible blood leading to an acute hemolytic reaction), many hospital transfusion services require confirmation of the patient's ABO type via a second sample prior to transfusion of type-specific RBCs or the transfusion of group O RBCs until the time of such confirmation.

- 3. What method would you use to perform compatibility testing for this patient based on the sample and test results of the second admission, and what would be the ABO/Rh type of the RBCs that you would crossmatch? What risk is involved?** During the second admission, RBCs may be crossmatched using any one of the three methods, though many blood banks would prefer the electronic crossmatch now that the patient's ABO type has been confirmed by testing of an additional sample (and matches the results of the first sample received during the prior admission), the antibody screen is negative, and there is no known history of clinically significant alloantibodies in the patient. This of course presumes that the blood bank has a computer system that has been validated to perform the electronic crossmatch function (most blood banks do have such systems in place), which involves a method to verify correct data entry before release of blood components and logic to alert the user to ABO group and Rh type discrepancies between the patient and the donor unit. Essentially, in the electronic crossmatch, the computer system verifies the patient's ABO type and selects an ABO-compatible donor unit from the available inventory in the blood bank refrigerator (blood donor unit inventory is logged into the computer system and contains the donation identification number, component name, and the ABO

group and Rh type of each unit), conveniently eliminating the need for a serologic crossmatch. Here again, ABO type-specific RBCs could be crossmatched for the patient, but the chief risk again is patient misidentification given that the patient has changed her last name since the time of the first admission; thus, some may prefer to obtain another sample on the second admission to confirm the ABO type or to crossmatch group O RBCs for the time being.

4. **What method would you use to perform compatibility testing for this patient based on the sample and test results of the third admission?** Testing of the sample from the third admission shows a positive antibody screen with an anti-E alloantibody identified on the panel. Thus, an AHG crossmatch must be performed against a donor RBC unit known to be negative for the Rh(E) antigen. Also, as a result of this history, even if the anti-E becomes undetectable in the future (i.e., evanescence), the patient must always receive Rh(E)-negative blood crossmatched by the AHG method (except, of course, in hemorrhagic emergency whereby uncrossmatched blood may need to be given).

Reference

1. Yazdanbakhsh K, Ware RE, Noizat-Pirenne F. Red blood cell alloimmunization in sickle cell disease: pathophysiology, risk factors, and transfusion management. *Blood*. 2012;120(3):528–37.

Recommended Reading

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Chapter 4

Cold Case



Clinical History

A 35-year-old woman with a history of prior Cesarean section (C-section) now presents at 39 weeks of gestation for repeat C-section. A sample (ethylenediamine-tetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank for type and screen along with an order for two units of red blood cells (RBCs). The patient has no history of prior transfusion.

ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	3+	0	4+
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	0	W+	NT	
SC2	0	0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Tube Panel

Cell #	Rh-hr	Rh-hr								Kell				Duffy		Kidd		Lewis	MNS			P	Lutheran	Test results: IAT/tube LISS									
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	IS	37 °C	AHG	CC	
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	+	+	+	0	0	0	0	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	0	0	0	+	+	0	+	0	0	0	0	0	0	0	0	2+
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	0	+	0	+	0	+	0	0	0	0	+	0	+	0	+	+	+ ^S	+	0	2+	0	W+	NT
4	R ₀ r	+	0	0	+	+	+	0	0	0	0	+	0	+	0	0	0	0	0	0	0	0	+	+	+	+	+	0	0	0	0	2+	
5	r' r	0	+	0	+	+	+	0	+	0	0	+	0	+	0	0	+	0	+	+	0	+	0	+	+	+	+	0	2+	0	W+	NT	
6	r'' r	0	0	+	+	+	+	0	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	+	+	+ ^w	+	0	0	0	0	2+	
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+ ^S	+	0	0	0	0	2+	
8	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	+	+	+	0	+	+	+	0	+	+	+	+ ^S	+	0	2+	0	W+	NT	
9	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	0	0	+	0	+	0	0	0	0	0	0	0	2+	
10	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	0	0	+	+	+	+	+ ^w	+	0	0	0	0	2+	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	+	0	2+	0	W+	NT	
Patient cell																													0	0	0	0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What is the patient's ABO/Rh type?
2. What antibodies did you identify?
3. What antibodies cannot be ruled out?
4. Are the antibodies clinically significant? Why or why not?
5. Is there a risk of hemolytic disease of the fetus/newborn (HDFN)? Why or why not?

Answers

1. **What is the patient's ABO/Rh blood type?** The patient is group A, Rh-positive blood type. Refer to Chap. 1, question 1 answer for further information on forward and reverse ABO typing.
2. **What antibodies did you identify?** Anti-Le^a (Lewis blood group) antibodies are present.
3. **What antibodies cannot be ruled out?** Anti-V and anti-Kp^a cannot be ruled out but are unlikely since these would react more strongly at warm temperatures.
4. **Are the antibodies clinically significant? Why or why not?** Anti-Le^a as well as anti-Le^b antibodies are not usually clinically significant since they are cold-reacting immunoglobulin (Ig)M antibodies (i.e., reactive at room temperature); they do not cause hemolytic transfusion reactions or hemolytic disease of the fetus/newborn. Lewis antibodies are typically found in Le(a-/b-) individuals. Warm-reacting IgG anti-Le^a has been found on rare occasions. It is not uncommon for some women who are Le^a- or Le^b-positive to lose the Lewis antigen expression during pregnancy and develop Lewis antibodies.
5. **Is there a risk of HDFN? Why or why not?** The baby is not at risk for HDFN because the Lewis antibodies are generally cold-reacting IgM; IgM is a pentameric molecule that is too big to cross the placenta unlike IgG which can breach the placental barrier (however, there is some evidence that IgG anti-Le^a capable of crossing the placenta is relatively common) [1]. Also, Lewis antigens are not RBC antigens per se; rather, they are plasma antigens that are adsorbed to the RBC membrane but are poorly expressed on neonatal RBCs; thus, Lewis antibodies are not a cause of HDFN.

Reference

1. Spitalnik S, Cowles J, Cox MT, Blumberg N. Detection of IgG anti-Lewis (a) in cord sera by kinetic Elisa. *Vox Sang.* 1985;48(4):235–8.

Recommended Reading

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Chapter 5

Buried Treasure



Clinical History

A 58-year-old man presents to the hospital for knee replacement surgery. The patient has a history of transfusion (at another hospital) some years ago after gastrointestinal bleeding. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	1+			
SC2	1+			

Reaction scale = 0 (no reaction) to 4 + (strong reaction)

Gel Panel

Cell #	Rh-hr	Rh-hr										Kell				Duffy		Kidd		Lewis			MNS			P	Lutheran		Test results
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	0	0	0	0	+	+	+	+	0	0	+	2+
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	+	0	+	2+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	+	0	0	+	+	+	+	0	+	W+
5	r' r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0
6	r'' r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	+	+	+	0	+	W+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	+	+	+	+	+	0	+	2+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	+	+	0	+	0
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	0	0	0	0	0	0	0	+	2+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	+	+	0	+	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	+	+	0	+	2+
Patient cell																		0	2+										0

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Tube Panel

		Rh-hr							Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube LISS								
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lr ^a	Lr ^b	4 °C	37 °C	AHG	CC	
1	R ₁ wR ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	4+	0	1+	NT
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	0	0	+	+	4+	0	1+	NT
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	0	0	+	+	^s	0	+	+	4+	0	1+	NT
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	+	0	0	+	+	+	+	+	0	+	4+	0	0	2+
5	r ⁺ r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	+	0	+	4+	0	0	2+
6	r ⁺ r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	+	^w	0	+	+	3+	0	0	2+
7	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	^s	0	+	+	4+	0	1+	NT
8	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	+	+	0	+	+	0	+	+	+	^s	0	+	+	3+	0	0	2+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	0	0	+	+	4+	0	1+	NT
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	+	^w	0	+	+	2+	0	1+	NT
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	0	+	+	0	+	4+	0	1+	NT
Patient cell																													3+	0	0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Selected-Cell (“Rule-out”) Panel

		Rh-hr						Kell					Duffy		Kidd	Lewis	MNS			P	Lutheran	Test results: IAT/tube LISS									
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	37 °C	AHG	CC
1	R ₀ W ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	0	2+	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	0	+	0	+	0	+	+	0	0	0	0	0	2+	
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	+	+	+	0	0	NT	
4	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	0	0	0	2+	
Patient cell																															

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak, mf mixed field

Questions

1. What antibodies did you identify?
2. Are the antibodies clinically significant? Why or why not?
3. What is the purpose of the selected-cell panel?
4. How many red blood cell (RBC) units would you need to screen in order to find two compatible (i.e., negative for the corresponding antigen) units in case the patient needs blood transfusion? (Refer to the Table of RBC Antigen Frequencies, using antigen frequencies listed under Caucasian population.)
5. Given that the homozygous Jk^a -antigen phenotype (Jk^{a+}/Jk^{b-}) in the Caucasian population is 26% and assuming Hardy–Weinberg equilibrium, calculate the frequency of the homozygous Jk^b -antigen phenotype (Jk^{a-}/Jk^{b+}) as well as the heterozygous phenotype (Jk^{a+}/Jk^{b+}) in the Caucasian population. Do the actual observed Kidd phenotypes follow Hardy–Weinberg equilibrium?

Answers

1. **What antibodies did you identify?** A cold autoantibody and an alloanti- Jk^a are evident.
2. **Are the antibodies clinically significant? Why or why not?** The anti- Jk^a antibody is clinically significant since it can cause hemolytic transfusion reactions and hemolytic disease of the fetus/newborn (HDFN). Although anti-Kidd antibodies are immunoglobulin (Ig)G, they can still activate complement and result in intravascular hemolysis. Unfortunately, they are notorious for becoming undetectable after some time (antibody evanescence); that is, the titer of the antibody drops below detectable levels. The cold autoantibody, on the other hand, is not clinically significant.
3. **What is the purpose of the selected-cell panel?** In this case, a selected-cell panel was run for the purpose of ruling out antibodies that could not otherwise be ruled out on the initial tube panel. Specifically, anti-C and anti-E could not be ruled out because there were no homozygous $Rh(C)$ and $Rh(E)$ cells that were also Jk^a -negative on the panel. Thus, only R_1R_1 and R_2R_2 , Jk^a -negative cells were tested on the selected-cell panel. Antibodies are typically ruled out on cells with homozygous antigen expression because of “dosage” effect. Basically, this means that weak or low-titer antibodies might not react with cells expressing only a single copy of the antigen (i.e., heterozygous antigen expression). Thus, whenever possible, it is best to rule out antibodies based on homozygous (“double-dose”) antigen expression (typically, this is true for the Rh, MNSs, Duffy [Fy^a/Fy^b], and Kidd [Jk^a/Jk^b] blood group antigens; the main exception is for Kell [unless anti-K is highly suspected] since it is very difficult to find cells that are K-antigen homozygous).

4. **How many RBC units would you need to screen in order to find two compatible (i.e., negative for the corresponding antigen) units in case the patient needs blood transfusion? (Refer to the Table of RBC Antigen Frequencies.)** Given that the frequency of Jk^a antigen is approximately 77% (Caucasian population frequency), there is a 23% chance of finding an RBC unit that is Jk^a -antigen negative. Thus, $2/0.23 = 8.69$ or 9 units need to be screened in order to find two that are Jk^a -antigen negative. Note that the cold autoantibody does not factor into this equation since it is not clinically significant and can be ignored for transfusion purposes. Also note that although there is a high probability that the patient may not need blood for his knee surgery, given the advanced surgical techniques used today to minimize blood loss, it is a good idea to prepare blood ahead of time for any patient who has an antibody problem since there is always the slightest chance of unexpected bleeding requiring transfusion.
5. **Given that the homozygous Jk^a -antigen phenotype (Jk^a+/Jk^b-) in the Caucasian population is 26% and assuming Hardy–Weinberg equilibrium, calculate the frequency of the homozygous Jk^b -antigen phenotype (Jk^a-/Jk^b+) as well as the heterozygous phenotype (Jk^a+/Jk^b+) in the Caucasian population. Do the actual observed Kidd phenotypes follow Hardy–Weinberg equilibrium?** The Hardy–Weinberg equation states that $p^2 + 2pq + q^2 = 1$ [1]. We are given that $p^2 = 0.26$ (26%) for the homozygous Jk^a phenotype in the Caucasian population [2]. Thus, $p = \sqrt{0.26} = 0.51$ and $q = 1 - p$ or $1 - 0.51 = 0.49$. Therefore, $q^2 = (0.49)^2 = 0.24$; so the frequency of homozygous $Jk^b = 24\%$. Then, to calculate the heterozygous (Jk^a+/Jk^b+) frequency, plugging in the numbers for $2pq = 2 \times 0.51 \times 0.49 = 0.50$ (with rounding). Thus, plugging in all numbers into the Hardy–Weinberg equation as a check, we get $0.26 + 0.50 + 0.24 = 1$. These calculated frequencies indeed match the reported frequencies [2]; note that Hardy–Weinberg equilibrium may not be followed in cases in which mutations occur, there is natural population selection, the population is too small, or mating is not random [1]. Finally, the astute reader will notice that the total Jk^a frequency calculated from the above equation ($p^2 + 2pq$) does not exactly match the frequency reported in the Table of RBC Antigen Frequencies; that is, $0.26 + 0.50 = 0.76$ (76%) vs. 77% in the table. This may perhaps be attributed to rounding (however, these Jk^a frequencies match those reported in sources [3]).

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Chapter 6

I Can “See” Clearly Now



Clinical History

A 16-year-old woman with sickle cell disease now presents with aplastic crisis with a hematocrit (Hct) of 17% (the patient's baseline Hct is 28%). The patient has been transfused many times with the last red blood cell (RBC) transfusion occurring 1 month ago. The patient has a known history of anti-E antibody. A sample (ethylene-diaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank for stat type and screen with request for two RBC units.

ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	3+	4+	4+
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	0	0	2+	
SC2	3+	4+	NT	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Tube Panel

Cell #	Rh-hr	Rh-hr							Kell				Duffy	Kidd	Lewis	MNS			P	Lutheran	Test results: IAT/tube LISS										
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a				Kp ^b	Js ^a	Js ^b			Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁
1	R ₁ wR ₁	+	+	0	0	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	0	0	0	2+	
2	R ₁ R ₁	+	+	0	0	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	0	0	0	2+	
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	0	+	0	+	0	0	0	0	0	0	0	0	0	+	+	+	+	0	3+	4+	NT
4	R ₀ r	+	0	0	+	+	0	+	0	0	0	+	0	+	0	0	0	0	0	0	0	0	+	+	+	+	0	1+	1+	NT	
5	r'r	0	+	0	+	+	0	0	0	0	0	+	0	+	0	0	0	0	0	+	0	+	0	+	+	+	0	W+	NT		
6	r''r	0	0	+	+	+	0	0	0	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	3+	4+	NT	
7	rr	0	0	0	+	+	0	0	0	+	0	+	0	+	0	+	0	0	+	0	+	0	+	+	+	+	0	1+	NT		
8	rr	0	0	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	1+	NT		
9	rr	0	0	0	+	+	0	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	0	1+	NT		
10	rr	0	0	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	1+	NT		
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	0	0	2+	
Patient cell		2+		mf																				0	W+	NT					

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak, mf mixed field

DAT		
Polyspecific:	1+	Anti-IgG: 1+
		Anti-C ₃ d: 0

Questions

1. What antibodies did you identify?
2. Are the antibodies clinically significant? Why or why not?
3. Can the weakly positive autocontrol and direct antiglobulin test (DAT) be explained? How does the Rh(c)-antigen phenotype result help you?
4. What antibodies would you expect to find in the eluate?

Answers

1. **What antibodies did you identify?** The patient has developed anti-c in addition to anti-E antibodies which are both identifiable on the panel. This scenario occurs as patients who develop anti-E antibodies are often exposed to the Rh(c) antigen. That is, when Rh(E)-antigen-negative cells are transfused, they may be Rh(c)-antigen-negative (R_1) or Rh(c)-antigen-positive (i.e., R_0 [Dce] or r [ce]), the latter eliciting anti-c antibodies. The converse is not true, however, since patients with anti-c antibodies are not likely to be exposed to RBCs carrying the Rh(E) antigen (i.e., the majority of c-negative RBCs are E-negative [recall that the R_z (DCE) and r^y (CE) haplotypes are uncommon]; refer to Chap. 2, question 3 answer for review of Rh haplotypes) [1, 2].
2. **Are the antibodies clinically significant? Why or why not?** Most Rh antibodies are clinically significant since they are warm-reacting (i.e., 37 °C) immunoglobulin (Ig)G antibodies capable of causing a delayed hemolytic transfusion reaction or hemolytic disease of the fetus/newborn (HDFN). After alloimmunization, antibodies may persist for many years. The Rh(c) antigen is more immunogenic than the Rh(E) antigen. Anti-c may cause severe HDFN; anti-E usually does not cause HDFN, and, when it does, it is usually mild (though severe cases have been reported) [3].
3. **Can the weakly positive autocontrol and DAT be explained? How does the Rh(c)-antigen phenotype result help you?** It is likely that anti-c antibody is causing the weakly positive autocontrol and DAT. From the Rh(c) phenotype (i.e., antigen typing) result, it is evident that the patient has a mixed population (i.e., mixed field) of RBCs in circulation due to the recent transfusion (1 month ago) and that the RBCs from at least one of the donor units is positive for Rh(c) antigen to which the patient has newly developed alloantibodies. In addition, phenotyping of the donor RBCs from tubing segments, if available (donor tubing segments are saved for a week or more after transfusion in case of reaction), could be performed to confirm Rh(c)-antigen-positive donor RBCs as the cause of the positive autocontrol in this patient.
4. **What antibodies would you expect to find in the eluate?** Anti-c antibodies are expected since they would be bound to the transfused Rh(c)-antigen-positive donor RBCs in the patient's circulation. Anti-E should not be bound to the

patient’s RBCs since the patient should have been receiving Rh(E)-antigen-negative RBCs based on the history of anti-E and, therefore, would not be eluted. Elution techniques release and concentrate antibodies that are bound to red cells; the resulting eluate can be tested against a panel to confirm the specificity of the antibody coating the RBCs.

References

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- Leger RM. The positive direct antiglobulin test and immune-mediated hemolysis. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff CM, editors. *Technical manual*. 18th ed. Bethesda: AABB; 2014. p. 425.

Chapter 7

You Really “Oughta” Get This



Clinical History

A 20-year-old woman with a history of systemic lupus erythematosus and idiopathic (immune) thrombocytopenic purpura (ITP), who received intravenous Rh immune globulin (IV RhIg) 1 year ago for the treatment of ITP and has no prior history of red blood cell (RBC) transfusion or pregnancy, is found to have a hematocrit (Hct) level of 15%. The patient was referred to the emergency department by her primary care physician for stat RBC transfusion of two units. A type and screen sample along with an order for the RBCs (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	4+	3+	0	0
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	0	0	2+	
SC2	W+	1 +	NT	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); W weak

Tube Panel

Cell #	Rh-hr	Rh-hr							Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube LISS								
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lr ^a	Lr ^b	37 °C	AHG	CC		
1	R ₁ wR ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	0	0	0	2+	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+	+	+	0	0	0	0	0	0	2+	
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	+	+	+	+	s ₊	0	+	W+	1+	NT	
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	+	0	+	W+	1+	NT	
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	+	+	+	0	+	W+	NT	NT	
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	+	+	w ₊	0	+	W+	1+	NT	
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	s ₊	0	+	W+	1+	NT	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	+	s ₊	0	+	W+	1+	NT	
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	0	0	+	W+	1+	NT	NT
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+	w ₊	0	+	W+	1+	NT	NT	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	+	+	+	0	0	0	0	2+	
Patient cell		2+	2+	2+	2+																							W+	1+	NT			

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

DAT profile		
Polyspecific:	1+	Anti-IgG: 1+
Anti-C ₃ d: 1+		

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Acid Eluate Panel

Cell #	Rh-hr	Rh-hr							Kell				Duffy	Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube PEG							
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a		Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b		Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	+	+	+	+	0	+	W+	NT
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	+	+	0	0	+	+	+	+	0	0	+	W+	NT	
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	0	+	+	+	+	S ₊	0	+	1+	NT
4	R ₀ r	+	0	0	+	+	0	+	0	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	+	0	+	1+	NT
5	r' r	0	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	+	0	+	W+	NT
6	r'' r	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	w ₊	0	+	1+	NT
7	rr	0	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	s ₊	0	+	1+	NT	
8	rr	0	0	0	+	+	0	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	0	s ₊	0	+	1+	NT	
9	rr	0	0	0	+	+	0	0	0	0	+	+	0	+	0	+	+	+	0	0	0	+	0	+	0	0	0	+	1+	NT	
10	rr	0	0	0	+	+	0	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+	w ₊	0	+	1+	NT	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	+	+	+	+	0	+	W+	NT
Last wash SC1																													0	2+	
Last wash SC2																													0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Autoadsorption Panel

		Rh-hr							Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube PEG							
Cell #	Rh-hr	D	C	E	c	e	f	c ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC		
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	0	2+	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	0	0	2+	
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	+	+	s ₊	0	+	0	0	2+	
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	+	0	+	0	2+	
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	+	+	+	0	+	0	2+	
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	w ₊	0	+	0	0	2+	
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	s ₊	0	+	0	0	2+	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	s ₊	0	+	0	0	2+	
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	0	0	+	0	0	+	+	0	0	0	+	0	0	2+	
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+	w ₊	0	+	0	0	2+	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	+	+	+	+	0	+	0	0	2+
Patient cell																														0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What antibodies did you identify?
2. What is the significance of the autocontrol and direct antiglobulin test (DAT) in this case?
3. What is the significance of the patient's Rh(c) phenotype, eluate panel, and autoadsorption panel results?
4. Write the patient's Rh phenotype using the Rh–hr (Wiener) system:
5. How would you manage this patient's RBC transfusion needs?
6. What is the mechanism by which IV RhIg improves ITP? What is the significance of IV RhIg treatment in this case, if any?
7. Suppose that the patient had received IV RhIg within the past month instead of 1 year ago, would anything about your RBC transfusion management change?

Answers

1. **What antibodies did you identify?** Warm autoantibody with anti-c specificity is present.
2. **What is the significance of the autocontrol and DAT in this case?** In the absence of recent transfusion (i.e., transfusion within prior 3 months), a positive autocontrol and DAT signify the presence of an autoantibody. It should be noted that a positive DAT alone does not signify hemolysis; rather, a workup for hemolysis must be performed, including tests for markers of hemolysis (e.g., bilirubin, lactate dehydrogenase [LDH], and haptoglobin [which are more typically markers for intravascular hemolysis]) and a review of the peripheral blood smear (which may, e.g., show spherocytosis as an indication of extravascular hemolysis). However, clinically significant hemolysis is more likely to occur when there is C₃d (complement) coating of the RBCs in addition to immunoglobulin (Ig)G (remember that the polyspecific DAT includes reactivity for both IgG and C₃d; note that for the purposes of this workbook, C₃d is used to denote complement fixation though some anticomplement reagents detect C₃b complement component ± C₃d). It should also be remembered that autoimmune hemolytic anemia can occur in the absence of a positive DAT, for example, when the level of bound IgG is below detection (i.e., less than 200 molecules of bound IgG per RBC) or when IgA antibodies are involved that are not detected by typical Coombs reagent. The overall annual incidence of warm autoimmune hemolytic anemia (WAIHA) is estimated to be 1 per 75,000–80,000 persons, affecting people of all ages [1].
3. **What is the significance of the patient's Rh(c) phenotype, eluate panel, and autoadsorption panel results?** The panel results show that the warm autoantibody has anti-c-like specificity. The eluate and autoadsorption panels help to confirm the presence of an autoantibody and rule out alloantibody presence.

Elution is a technique that removes and concentrates IgG antibody bound to the patient's RBCs, allowing for identification of the removed antibody. Autoadsorption, on the other hand, uses autologous RBCs to adsorb warm autoantibodies from plasma, allowing for identification of any present alloantibodies left behind. A major limitation of autoadsorption is that it should not be used in a recently transfused (i.e., within prior 3 months) patient as circulating donor RBCs may adsorb out alloantibodies in addition to autoantibodies, thus leading to a false-negative result. Also, note that while the autoadsorption panel in this particular case is “clean” (i.e., the autoantibodies are entirely adsorbed out), in some cases, autoantibodies may not be entirely removed owing to technique or to a high titer of the autoantibodies.

4. **Write the patient's Rh phenotype using the Rh–hr (Wiener) system:** Given that the patient is positive for all five of the major Rh antigens (DCcEe), the most likely phenotype is R₁R₂. If you do not recall Rh nomenclature, this is a good time to review the Wiener and Fisher–Race Rh nomenclature. See table in Chap. 2, question 3 answer.
5. **How would you manage this patient's RBC transfusion needs?** The patient should be transfused conservatively (i.e., only in the event of symptomatic anemia) since warm autoantibodies can mask underlying alloantibodies which may develop from an exposure to antigens on donor RBCs. Medical treatment of the WAIHA with steroid or other immunosuppressive agent (intravenous immunoglobulin [IVIG], rituximab [monoclonal anti-CD20/B-cell marker antibody]) should be started promptly; splenectomy may be used for very severe refractory cases of WAIHA. Therapeutic plasma exchange has also been used for treatment of severe WAIHA [2], though it is listed as a category III indication (optimum role of apheresis not established) by the American Society for Apheresis (ASFA) [3]. If RBC transfusion is necessary, R₁R₁ (c-antigen negative) RBCs may be considered, though survival of such transfused RBCs may not be increased. In general, the term “least incompatible” is applied to RBCs transfused in the presence of warm autoantibodies, though this term is disliked by some immunohematologists [4].
6. **What is the mechanism by which IV RhIg improves ITP? What is the significance of IV RhIg treatment in this case, if any?** IV RhIg may be used for the treatment of ITP in patients who are Rh-positive and who have a functional spleen. The treatment works by inducing extravascular hemolysis (i.e., in the spleen) of IgG-coated Rh(D)-antigen-positive RBCs (thus blocking splenic Fc receptors) preferentially leaving IgG-coated platelets to circulate and raise the platelet count. Essentially, an iatrogenic autoimmune hemolytic anemia is created; thus, treatment consideration must take into account the patient's hemoglobin (Hgb) or Hct level such that caution should be taken with patients who have Hgb < 10 g/dL when treating ITP with IV RhIg. In this case, the history of IV RhIg treatment 1 year ago bears no significance since the administered antibodies would not circulate for more than 12 weeks. It is important to note though that further treatment with IV RhIg at this time would be contraindicated because of the patient's WAIHA and severe anemia.

7. **Suppose that the patient had received IV RhIg within the past month instead of 1 year ago, would anything about your RBC transfusion management change?** Unusually, this situation would result in transfusion of Rh(D)-negative RBCs to this Rh(D)-positive patient due to passively-acquired anti-D antibodies from the IV RhIg circulating in the patient.

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Chapter 8

What the Kell



Clinical History

A 66-year-old man is scheduled for an elective hip replacement surgery. A preadmission test sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank for type and screen along with an order for two units of red blood cells (RBCs). No transfusion history is provided.

ABO/Rh/Antibody Screen

ABO/Rh (tube method)				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	3+	1+	4+
Anti-A ₁ lectin	Patient: 4+	A ₁ cells: 4+	A ₂ cells: 0	
Antibody screen (tube LISS method)				
	37 °C	AHG	CC	
SC1	0	W+	NT	
SC2	0	2+	NT	

Reaction scale = 0 (no reaction) to 4 + (strong reaction); W weak

Tube Panel

Cell #	Rh-hr	Rh-hr							Kell				Duffy	Kidd	Lewis	MNS			P	Lutheran		Test results: IAT/tube LISS									
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a				Kp ^b	Js ^a	Js ^b		Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lr ^a
1	R _{1w} R ₁	+	+	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	2+	4+	0	1+	NT
2	R ₁ R ₁	+	+	0	0	+	0	0	+	0	0	+	0	+	+	+	+	+	0	+	0	+	0	+	0	+	2+	3+	0	2+	NT
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	0	+	0	+	0	0	0	0	+	0	0	0	+	+	+	+	2+	3+	0	W+	NT
4	R ₀ r	+	0	0	+	+	+	0	+	0	0	+	0	+	0	0	0	0	+	0	0	0	+	+	+	+	1+	3+	0	W+	NT
5	r'r	0	+	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	2+	3+	0	W+	NT
6	r''r	0	0	+	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	2+	3+	0	1+	NT
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	0	0	+	+	0	0	+	+	+	+	2+	3+	0	2+	NT	
8	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	2+	4+	0	1+	NT	
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	3+	3+	0	W+	NT
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	+	1+	3+	0	W+	NT	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	2+	3+	0	W+	NT
Patient cell																											2+	3+	0	W+	NT

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Pre-Warm Panel (37 °C)

		Rh-hr								Kell				Duffy	Kidd		Lewis		MNS				P	Lutheran	Test results: IAT/ tube LISS								
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b		Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b			M	N	S	s	P ₁	Lu ^a	Lu ^b	37 °C	AHG
Cell #	Rh-hr																																
1	R _{1w} R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+	+	0	0	0	0	2+	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	+	0	+	+	+	0	0	0	0	0	2+	NT		
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	0	+	0	0	+	0	+	s ₊	0	0	0	0	2+		
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	+	0	0	0	0	2+		
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	0	0	+	+	+	0	0	0	0	2+		
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	w ₊	0	0	0	0	0	2+		
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	+	s ₊	0	0	0	2+	NT			
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	s ₊	0	0	0	0	0	2+		
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	0	0	0	2+		
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	w ₊	0	0	0	0	0	2+		
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	0	0	0	+	0	+	0	+	0	0	0	0	0	0	2+	
Patient cell																												0	0	0	0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Additional Study: 4 °C Incubation (Cold Panel)

A ₁ cells	1+
O cells	4+
O cord cells	0
O _i cells	0

Questions

1. What antibodies did you identify?
2. How does the pre-warm panel help you?
3. What is the significance of the anti-A₁ lectin result?
4. How do you interpret the results of the 4 °C incubation panel using A₁ cells, O cells, O cord cells, and O_i cells? Do the results of this cold panel have any clinical significance?
5. How many donor RBC units need to be screened in order to find two compatible units as requested? (Refer to the Table of RBC Antigen Frequencies, using antigen frequencies listed under Caucasian population.)
6. What do the antibody findings suggest about this patient's past medical history?

Answers

1. **What antibodies did you identify?** Cold autoantibody and anti-K alloantibody are present.
2. **How does the pre-warm panel help you?** The pre-warm panel eliminates cold reactions allowing for identification of the warm anti-K antibody. Other methods to negate cold antibody reactions include cold autoadsorption and the use of rabbit erythrocyte stroma (RESt), both of which can remove the cold antibodies (typically immunoglobulin [Ig]M, anti-I, or anti-IH). RESt is an older technique not used often anymore and may remove anti-B antibodies as well.
3. **What is the significance of the anti-A₁ lectin result?** There is 1+ reactivity with A₁ cells in the patient's back type; this could be due to either a naturally occurring anti-A₁ antibody (if patient is of A₂ or other weak subtype of A) or interference from a cold-reacting antibody. The positive reaction with anti-A₁ lectin (*Dolichos biflorus*) indicates that the patient is A₁ type; thus, the reaction with A₁ cells in the reverse typing is not due to anti-A₁. In this case, it is due to cold autoantibody since the autocontrol is positive in the panel at cold temperatures.

4. **How do you interpret the results of the 4 °C incubation panel using A₁ cells, O cells, O cord cells, and O_i cells? Do the results of this cold panel have any clinical significance?** This identifies that the cold autoantibody is likely anti-IH (i.e., having reactivity with I and H antigens, I antigen is not expressed on cord cells and O_i cells, while H antigen is only weakly expressed on A₁ cells). The results of this panel, though, do not have any clinical significance. Cold-reacting antibodies are most commonly benign cold agglutinins; their significance lies in the potential for interference with ABO typing since this is generally performed at room temperature.
5. **How many donor RBC units need to be screened in order to find two compatible units as requested? (Refer to the Table of RBC Antigen Frequencies.)** K antigen, part of the Kell blood group antigen system, is present in only about 9% of the population (Caucasian frequency); thus, more than 90% of donor RBCs will lack the K antigen and will be compatible with the patient in this case. Dividing 2 by 0.9 equals 2.22; thus, only two units need to be screened in all probability to find two K-antigen-negative RBC units for the patient.
6. **What do the antibody findings suggest about this patient's past medical history?** The finding of anti-K antibody, a warm, immune alloantibody (i.e., requires prior exposure to the antigen for development), suggests that the patient received blood transfusion sometime in the past. Naturally occurring anti-K has been described, but in most cases is IgM, reacting best at room temperature and sometimes associated with infectious illness [1, 2]. Rare cases of autoanti-K mimicking an alloantibody have also been described [3]. If the patient denies any history of red cell transfusion, passive acquisition is possible (such as through recent plasma or platelet transfusion or, in the case of a woman, through exposure during pregnancy). Anti-IH is commonly seen in the serum of healthy A₁ individuals and does not imply any clinical history.

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Chapter 9

EeeeeK!!!



Clinical History

A 36-year-old woman with rheumatoid arthritis is found to have a hematocrit (Hct) of 22% on a routine clinic visit. She has a history of blood transfusion 3 years ago with a negative antibody screen at that time. The patient is referred to the outpatient transfusion service for transfusion of two units of red blood cells (RBCs); a type and screen sample along with an order for the RBCs (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	3+	4+	4+
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	2+	3+	NT	
SC2	2+	3+	NT	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Tube Panel

		Rh-hr							Kell				Duffy	Kidd	Lewis	MNS			P	Lutheran	Test results: IAT/tube LISS											
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	37 °C	AHG	CC	
1	R _W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	2+	3+	NT
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	0	0	+	+	2+	3+	NT
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	s ₊	0	+	0	W+	NT	
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	+	0	+	2+	3+	NT	
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	+	+	0	+	2+	3+	NT	
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	0	0	+	0	+	+	+	+	w ₊	0	+	2+	3+	NT	
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	0	0	+	+	+	s ₊	0	+	2+	3+	NT	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	s ₊	0	+	2+	3+	NT	
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	0	0	0	+	2+	3+	NT	
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	w ₊	0	+	2+	3+	NT		
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	0	0	+	0	+	2+	3+	NT
Patient cell		2+		0	0	2+																								2+	3+	NT

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

DAT profile		
Polyspecific: 3+	Anti-IgG: 3+	Anti-C ₃ d: 3+

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Autoadsorption Panel

		Rh-hr							Kell				Duffy		Kidd		Lewis		MNS				P		Lutheran		Test results: IAT/tube LISS					
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P _I	Lu ^a	Lu ^b	AHG	CC		
Cell #	Rh-hr	+	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	+	W+	NT
1	R ₁ wR ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	0	+	+	0	0	0	+	+	+	+	0	0	0	+	+	2+	NT
2	R ₁ R ₁	+	0	+	0	0	0	0	0	0	0	+	0	+	0	0	+	+	0	0	0	0	+	+	+	+	+	0	+	+	0	2+
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	0	+	0	+	0	0	0	+	+	0	0	0	+	+	+	+	+	+	+	+	W+	NT
4	R ₀ r	+	0	0	+	+	0	+	0	+	0	+	0	+	0	0	0	+	+	0	0	0	+	+	+	+	+	0	+	+	W+	NT
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	0	+	W+	NT
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	+	+	+	+	0	+	3+	NT
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	+	0	+	W+	NT	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	+	+	0	+	2+	NT	
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	+	0	+	0	0	0	+	0	+	0	0	0	+	W+	NT	
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+	+	+	0	+	3+	NT	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	0	+	W+	NT	
Patient cell																														W+	NT	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What antibodies did you identify?
2. How does the autoadsorption panel help you? What are the limitations of autoadsorption?
3. Can the differences in reaction strengths (i.e., W+, 2+ vs. 3+ reactions) on the adsorbed panel be explained?
4. Why was the direct antiglobulin test (DAT) profile performed and what is the significance of the results?
5. How would you manage this patient's anemia?
6. What antigens would you screen for prior to crossmatching donor RBCs for this patient? Does the patient's Rh(e)-antigen phenotype play any role in your decision?
7. What step might be taken when performing compatibility testing (i.e., cross-matching) for this patient?
8. Prior to transfusing this patient, what other testing should be done to facilitate detection of clinically significant alloantibodies that the patient may develop later on?

Answers

1. **What antibodies did you identify?** Warm autoantibody with anti-e-like specificity and alloanti-Fy^a antibodies are present. However, it should be noted that anti-e-like antibody may be difficult to distinguish from another related rare alloantibody, anti-hr^B (which may actually look like anti-Ce), and may be found in Rh(e)-variant phenotypes (usually African American individuals), though it is generally not clinically significant [1].
2. **How does the autoadsorption panel help you? What are the limitations of autoadsorption?** The adsorbed panel helps to uncover underlying alloantibody by removing autoantibody. The major limitation is that the autoadsorption technique cannot be used if the patient was recently transfused (i.e., RBC transfusion within the previous 3 months) since the presence of alloantigens on circulating donor RBCs may lead to a false-negative result by the removal of corresponding alloantibodies as well as autoantibodies. Also, autoadsorption is technically limited when the patient is severely anemic such that few RBCs are present to adsorb autoantibodies. Autoadsorption is often accomplished through the use of ZZAP [2] reagent (which combines papain with dithiothreitol, DTT) to dissociate immunoglobulin (Ig)G autoantibodies and also enhance adsorption of the autoantibodies from the plasma or serum; one must be aware, however, that ZZAP reagent destroys certain antigens, such as Duffy, Kell, and MNS.
3. **Can the differences in reaction strengths (i.e., W+, 2+ vs. 3+ reactions) on the adsorbed panel be explained?** The W+ reactions on the adsorbed panel are

consistent with baseline remaining unadsorbed autoantibody since the autocontrol reaction is W+ (i.e., not all of the autoantibody could be removed; compare this with the “clean” autoadsorption panel seen in Chap. 7).

Note that the R₂R₂ cell (cell #3) lacking Rh(e) antigen reacts weaker than other cells, consistent with anti-e-like specificity of the warm autoantibody in this case. The difference between the 2+ and 3+ reactions is due to dosage effect (see Chap. 5, question 3 answer) of the anti-Fy^a; homozygous expression of Fy^a antigen results in a stronger reaction strength than heterozygous (Fy^a/Fy^b) expression.

4. **Why was the DAT profile performed and what is the significance of the results?** The DAT profile, consisting of polyspecific, IgG, and C₃d DAT, was performed because the autocontrol was positive in the panel, indicating the possibility of an autoantibody.
5. **How would you manage this patient's anemia?** Since the patient's anemia was found incidentally on a routine clinic visit during the follow-up of her chronic condition (rheumatoid arthritis), it can be presumed (though careful history and assessment, of course, are advised) that the patient is not acutely symptomatic from the anemia. Thus, transfusion should be avoided, and the warm autoimmune hemolytic anemia (WAIHA) may be managed by conservative nontransfusion management (i.e., steroids or other immunosuppressive medications). Transfusion may be indicated as the patient becomes symptomatic from the anemia (e.g., shortness of breath, orthostatic changes, tachycardia, and hypotension). Refer to Chap. 7, question 5 answer for more information about the treatment of WAIHA.
6. **What antigens would you screen for prior to crossmatching donor RBCs for this patient? Does the patient's Rh(e)-antigen phenotype play any role in your decision?** Fy^a-negative RBCs are necessary for this patient. It may also become necessary to give Rh(e)-negative (R₂) cells to the patient due to the anti-e-like specificity of the warm autoantibody in case of poor transfusion response to transfused R₁ cells. Rh(e)-negative cells, however, are rare and will have limited availability (see Table of RBC Antigen Frequencies in front section of the workbook).
7. **What step might be taken when performing compatibility testing (i.e., cross-matching) for this patient?** The use of the adsorbed serum, if available after testing on the panel, can be used for crossmatching to reduce incompatibility with the warm autoantibody. Though the donor RBCs may appear to be “compatible” in vitro using the adsorbed serum, once transfused, the donor cells will be hemolyzed by the autoantibody. The term “least incompatible” is often used (though disliked by immunohematologists) to indicate a compatibility reaction strength not greater than the autocontrol reaction and, thus, the most compatible donor cells for the patient with warm autoantibody [3].
8. **Prior to transfusing this patient, what other testing should be done to facilitate detection of clinically significant alloantibodies that the patient may develop later on?** The patient's RBCs should be phenotyped for significant antigens (i.e., Rh, Kell, Duffy, Kidd). This will help to determine which antigens the

patient's RBCs lack and, thus, what antibodies the patient may make in the future. However, phenotyping of some antigens may be limited due to the interference caused by coating of the RBCs with IgG warm autoantibody. Under such circumstances, antigens may only be accurately phenotyped if the antisera reagents are monoclonal (i.e., IgM-based, which are not affected by IgG coating of RBCs) or if the IgG-coated RBCs are first treated using a technique to remove the IgG while leaving RBC antigens intact. RBC phenotyping is of limited to no value if the patient was recently (i.e., within the prior 3 months) transfused due to the presence of circulating donor RBCs causing false-positive (i.e., mixed field) results; in such cases, molecular typing may be useful (i.e., a predicted RBC antigen phenotype may be elucidated via genotyping). Molecular-predictive red cell phenotyping is increasingly being used in clinical settings as an adjunct to classical serologic immunohematology.

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Chapter 10

Are You Kidding?



Clinical History

A 72-year-old man with myelodysplastic syndrome, multiply transfused with a known history of anti-E and anti-c antibodies, presents to the outpatient transfusion service. He was transfused with two units of red blood cells (RBCs) 5 weeks ago. The patient's hematocrit (Hct) is now 22% and one unit of RBCs is requested for the patient. A type and screen sample along with an order for a unit of RBCs (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	4+	3+	4+	0
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	0	3+	NT	
SC2	3+	4+	NT	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Tube Panel

		Rh-hr							Kell				Duffy	Kidd	Lewis		MNS			P	Lutheran		Test results: IAT/tube LISS									
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	37 °C	AHG	CC	
1	R ₀ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	2+	NT	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	0	3+	NT	
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	+	+	+	s ₊	0	+	4+	NT	
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	0	+	1+	3+	NT	
5	r ⁺ r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	0	+	1+	2+	NT	
6	r ⁺ r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	w ₊	0	+	4+	NT		
7	r	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	0	0	+	0	+	s ₊	0	+	1+	3+	NT	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	+	s ₊	0	+	1+	2+	NT	
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	1+	3+	NT		
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	0	+	+	+	+	w ₊	0	+	1+	3+	NT	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	+	0	+	0	+	0	3+	NT
Patient cell																		mf	2+									1+	2+	NT		

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak, mf mixed field

DAT profile		
Polyspecific: 3+	Anti-IgG: 3+	Anti-C ₃ d: 0

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Selected-Cell (“Rule-Out”) Panel

		Rh-hr								Kell				Duffy		Kidd	Lewis		MNS			P	Lutheran	Test results: IAT/tube LISS							
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	37 °C	AHG	CC
1	R ₁ R ₁	+	+	0	0	0	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	0	2+
2	R ₁ R ₁	+	+	0	0	0	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	0	0	2+
3	R ₁ R ₁	+	+	0	0	0	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	0	0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Acid Eluate Panel

		Rh-hr								Kell				Duffy		Kidd		Lewis		MNS				P	Lutheran		Test results: IAT/tube					
		Cell #	Rh-hr	D	C	E	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M		N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC
1	R _W R ₁	+	+	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	2+	2+	NT
2	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	0	+	3+	3+	NT
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	+	0	+	s ₊	0	+	3+	3+	NT
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	+	0	+	3+	3+	NT
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	+	0	2+	2+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	+	+	w ₊	0	+	0	2+	2+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	s ₊	0	+	2+	2+	NT
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	s ₊	0	+	0	2+	2+
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	+	0	0	+	3+	3+	NT
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	+	+	w ₊	0	+	3+	3+	NT
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	0	0	+	+	0	+	+	0	+	3+	3+	NT
Last wash SC1																														0	2+	
Last wash SC2																														0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What antibodies did you identify?
2. What is the cause of the positive direct antiglobulin test (DAT) result in this case? Does the mixed-field (mf) result for Jk^a antigen typing of the patient's sample provide evidence of the cause of the positive DAT?
3. How does the selected-cell panel help you?
4. How does the eluate panel help you?
5. Can the differences in reaction strengths (i.e., 2+ vs. 3+ reactions) on the eluate panel be explained?
6. What is the significance of the last wash results on the eluate panel using screening cells 1 and 2 (SC1 and SC2)?
7. In consideration of the patient's positive DAT result, would an autoadsorption panel be of help? Why or why not?
8. How many donor RBC units would you need to screen in order to find one compatible unit as requested? (Refer to the Table of RBC Antigen Frequencies, using antigen frequencies listed under Caucasian population.)

Answers

1. **What antibodies did you identify?** Anti-E and anti-c antibodies, known by history, are present in the panel; anti-Jk^a is evident from the eluate panel.
2. **What is the cause of the positive DAT result in this case? Does the mixed-field (mf) result for Jk^a antigen typing of the patient's sample provide evidence of the cause of the positive DAT?** The positive immunoglobulin (Ig)G DAT result is caused by the newly developed anti-Jk^a coating circulating transfused donor RBCs (the patient was transfused 5 weeks ago by history). The mixed-field result indicates that there are two populations of RBCs: one positive for the Jk^a antigen (transfused donor cells) and the other negative for the antigen (patient's cells).
3. **How does the selected-cell panel help you?** Since all cells on the initial panel are reactive, it is difficult to rule out antibodies. However, by selecting panel cells with phenotypes that are negative for antigens for which the patient has a known antibody history (i.e., anti-E and anti-c) or for which an antibody may be suspected (anti-Jk^a, based on the initial panel), other antibody specificities can be excluded. In this case, based on the selected panel cells, which are all Rh(E)-, Rh(c)-, and Jk^a-antigen negative, one can exclude the presence of other warm-reacting alloantibodies, particularly anti-K, anti-Kp^a, anti-Js^b, anti-Fy^a, anti-Fy^b, anti-Jk^b, anti-M, anti-S, anti-s, and anti-Lu^b antibodies.
4. **How does the eluate panel help you?** The eluate panel confirms that the positive DAT result is caused by the anti-Jk^a. Given that the patient has a known history of anti-E and -c alloantibodies, one would not expect to find these antibodies

in the eluate as the patient would have been transfused with compatible RBCs negative for these antigens.

5. **Can the differences in reaction strengths (i.e., 2+ vs. 3+ reactions) on the eluate panel be explained?** The different reaction strengths are due to a dosage effect between homozygous Jk^a and heterozygous Jk^a cells.
6. **What is the significance of the last wash results on the eluate panel using SC1 and SC2?** Last washes are negative controls to show that all bound antibodies have been eluted (i.e., removed) from the RBCs. Thus, last wash reactions should be negative as in this case. Positive last wash reactions indicate that antibody is still bound to the RBCs and that additional elution cycles should be performed to increase the antibody yield of the eluate; this will maximize the sensitivity of the eluate test in determining antibody specificity.
7. **In consideration of the patient's positive DAT result, would an autoadsorption panel be of help? Why or why not?** An autoadsorption panel is helpful when trying to identify alloantibodies that may be masked by the presence of a warm autoantibody. In this case, a warm autoantibody is not present (i.e., not the cause of the positive DAT result). Also, adsorption panels are technically difficult when the patient has been recently transfused (i.e., transfusion within the prior 3 months), as in this case, since circulating donor RBCs can adsorb alloantibody in addition to autoantibody causing a misleading negative panel (i.e., no alloantibody identified even though it is present).
8. **How many donor RBC units would you need to screen in order to find one compatible unit as requested? (Refer to the Table of RBC Antigen Frequencies.)** The patient requires donor RBCs that lack Rh(E), Rh(c), and Jk^a antigens. Statistically, 70% of the Caucasian population will be Rh(E)-antigen negative, 20% Rh(c)-antigen negative, and 23% Jk^a-antigen negative. Dividing 1 unit by $(0.7 \times 0.2 \times 0.23) = 31$ units.

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Chapter 11

G-Force



Clinical History

A 26-year-old woman presents at 28 weeks of gestation for Rh immune globulin (RhIg) injection. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	0	4+	4+
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	2+	3+	NT	
SC2	2+	3+	NT	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Tube Panel

Cell#	Rh-hr	Rh-hr							Kell				Duffy	Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube LISS								
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a		Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b		Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	37 °C	AHG
1	R ₀ W ₁	+	+	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	2+	3+	NT
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	0	0	+	0	+	+	+	0	0	0	+	+	+	+	0	0	+	2+	3+	NT	
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	+	0	+	+	+	0	+	2+	3+	NT
4	R ₀ r	+	0	0	+	+	+	0	+	0	0	+	0	+	0	0	0	0	0	0	0	0	+	+	+	+	0	+	2+	3+	NT	
5	r'r	0	+	0	+	+	+	0	0	0	0	+	0	+	0	0	+	0	+	+	0	0	+	+	+	+	0	+	2+	3+	NT	
6	r''r	0	0	+	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	0	0	2+
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	0	+	0	0	2+
8	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	+	0	+	0	0	+	+	+	+	+	0	+	0	0	2+
9	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	0	0	0	2+
10	rr ⁶	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	+	+	+	+	+	0	+	2+	3+	NT
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	+	+	0	+	+	0	+	2+	3+	NT
Patient cell																														0	0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak
*Additional panel information: cell #10 r^G (G antigen-positive)

Antibody titration	
Rh(D) antibody titer*: 32	Rh(C) antibody titer*: 32

*Using R₂ cell (panel cell #3)
**Using r' cell (panel cell #5)

Neonatal Results

A healthy baby boy is delivered at 39 weeks.

Test Results: Cord Blood

ABO/Rh (tube method)			
Cord RBCs			
Anti-A	Anti-B	Anti-D	Weak D
3+	0	0	0
Cord anti-IgG DAT: 2+			
Reaction scale = 0 (no reaction) to 4+ (strong reaction)			

Acid Eluate Panel (Baby's Cells)

		Rh-hr				Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube PEG								
Cell #	Rh-hr	D	C	E	e	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P _I	Lu ^a	Lu ^b	AHG	CC
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	0	+	2+
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	0	+	0	+	+	+	0	+	2+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	+	0	+	2+
5	r' r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	0	+	+	0	0	+	+	+	+	0	+	2+
6	r'' r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	0	+	0	+	+	+	+	+	+	0	+	2+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	0	+	2+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	0	0	0	+	+	+	+	0	+	2+	
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	+	0	0	+	0	0	0	0	+	2+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	+	+	+	0	+	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	0	+	2+
Last wash SC1																													0	2+
Last wash SC2																													0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What is the mother’s ABO/Rh type?
2. What possible antibodies are evident in the mother from the panel results?
3. In light of the anti-D titer and in consideration of the panel findings, is the mother a candidate for prenatal RhIg? Would antibody titration using the r^G cell (panel cell #10) be of any help in your decision? Why or why not?
4. What is the baby’s ABO/Rh type?
5. What is the most likely cause of the baby’s positive direct antiglobulin test (DAT) result?
6. What additional testing should be done to confirm the cause of the baby’s positive DAT?
7. Should the mother receive postpartum RhIg? Why or why not?

Answers

1. **What is the mother’s ABO/Rh type?** The mother is group O-negative. See Chap. 1, question 1 answer for explanation.
2. **What possible antibodies are evident in the mother from the panel results?**
The panel shows apparent antibody specificity to Rh(D) and Rh(C) antigens. Given that the r^G cell (cell #10: D–, C–, G+) is reactive with the mother’s serum, anti-G antibody is present. G antigen is present on all RBCs with Rh(D) and/or Rh(C) antigen; occasionally, G antigen is present on RBCs lacking Rh(D) and Rh(C) antigens (i.e., r^G cells) as in cell #10 on the panel. Additional testing may be done to rule out the presence of anti-D and anti-C in this case (double adsorption–elution) [1], though such testing may be technically difficult in the presence of anti-G (the table below may help to visually clarify antibody testing to discern anti-D, anti-C, and anti-G antibodies).

		Reagent				
Patient’s RBC antigens		Anti-D	Anti-C		Anti-G	
D+ C+ G+ (R ₁)		+	+		+	
D+ C– G+ (R ₀)		+	0		+	
D– C+ G+ (r’)		0	+		+	
D– C– G– (r)		0	0		0	
D– C– G+ (r ^G)		0	0		+	
Patient’s plasma antibodies		Anti-D Anti-C	Anti-D Anti-G	Anti-C Anti-G	Anti-D Anti-C Anti-G	Anti-G
D+ C– G+ (R ₀)	Adsorbed RBC eluate	Anti-D	Anti-D Anti-G	Anti-G	Anti-D Anti-G	Anti-G
	Adsorbed plasma	Anti-C	0	Anti-C	Anti-C	0

Patient's RBC antigens		Reagent				
		Anti-D	Anti-C		Anti-G	
D– C+ G+ (r')	Adsorbed RBC eluate	Anti-C	Anti-G	Anti-C Anti-G	Anti-C Anti-G	Anti-G
	Adsorbed plasma	Anti-D	Anti-D	0	Anti-D	0
D– C– G+ (r^G)	Adsorbed RBC eluate	0	Anti-G	Anti-G	Anti-G	Anti-G
	Adsorbed plasma	Anti-D Anti-C	Anti-D	Anti-C	Anti-D Anti-C	0

Reaction scale = 0 (no reaction) to + (positive)

- In light of the anti-D titer and in consideration of the panel findings, is the mother a candidate for prenatal RhIg? Would antibody titration using the r^G cell (panel cell #10) be of any help in your decision? Why or why not?**
Given that anti-G is present, the reported antibody titer may solely reflect that of the G antibody, and, in fact, anti-D may not even be present. Thus, since the mother is Rh-negative, a standard prenatal 300 µg dose of RhIg should be administered at 28 weeks of gestation. However, titration of the antibody using the r^G cell could be helpful in that if the titer result is about the same as that for the R₂ and r' cells, then the titer most likely reflects anti-G alone (without anti-D), whereas a significantly lower titer with the r^G cell may be suggestive of the presence of anti-D such that RhIg would not be indicated.
- What is the baby's ABO/Rh type?** The baby is group A-negative. Note that in testing the baby's ABO type, a reverse grouping is not performed as A and B isoantibodies do not develop until several months after birth, and any anti-A or anti-B present in the baby would be from the mother (i.e., maternal immunoglobulin [Ig]G anti-A and/or anti-B crossing the placenta). A test for weak D (indirect antiglobulin test [IAT] method) is performed to confirm that the baby is truly Rh-negative.
- What is the most likely cause of the baby's positive DAT result?** The positive DAT result could be due to anti-D, anti-C, and/or anti-G antibodies, since these were the antibodies that were identified or could not be excluded in the mother. However, anti-D, anti-C, and anti-G are all excluded as the cause since the eluate panel is negative; in addition, anti-D is excluded since the baby is Rh-negative. Therefore, given that the mother is group O type while the baby is group A type, anti-A₁ is the most likely cause of the baby's positive DAT. Group O mothers commonly produce IgG-class anti-A which can cross the placenta and coat group A fetal RBCs, causing hemolytic disease of the fetus/newborn (HDFN). Anti-A in the eluate would not react with group O panel cells.
- What additional testing should be done to confirm the cause of the baby's positive DAT?** The eluate should be tested with groups A and B RBCs to confirm anti-A₁ as the cause of the positive DAT in the baby.
- Should the mother receive postpartum RhIg? Why or why not?** The mother is not a candidate for postpartum RhIg since the baby is Rh-negative.

Reference

1. Yousuf R, Mustafa AN, Ho S-L, Tang Y-L, Leong C-F. Anti-G with concomitant anti-C and anti-D: a case report in a pregnant woman. *Asian J Transfus Sci.* 2017;11(1):62–4.

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Chapter 12

Hide and Seek



Clinical History

A 70-year-old man with myelodysplastic syndrome presents for outpatient transfusion. His hemoglobin (Hgb) level is 6.7 g/dL. The patient received two units of red blood cells (RBCs) 2 months ago, and the antibody screen was negative at that time. A type and crossmatch sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank; two units of RBCs are requested for the patient.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	4+	0	4+	0
<i>Antibody screen (AHG/gel method)</i>				
SC1	W+			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction); W weak

Gel Panel

		Rh-hr										Kell				Duffy				Kidd		Lewis		MNS				P	Lutheran		Test results		
Cell #	Rh-hr	D	C	E	e	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	M	N	S	P ₁	Lu ^a	Lu ^b	AHG gel	
1	R _W R ₁	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	+	0	
2	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	0	+	W+	
3	R ₂ R ₂	+	0	+	0	+	0	0	0	0	0	+	0	+	0	+	0	0	+	0	0	0	0	+	0	+	+	0	+	+	0	+	W+
4	R ₀ r	+	0	0	+	+	+	0	+	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	0	+	0	+	0	
5	r'r	0	+	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	+	0	0	+	0	+	0	
6	r''r	0	0	+	+	+	+	0	0	0	0	+	0	+	0	+	+	0	0	0	+	0	0	+	+	+	+	0	+	0	+	0	
7	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	0	+	+	0	0	0	+	+	+	+	0	+	+	0	+	0
8	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	+	0	+	+	0	+	0
9	rr	0	0	0	+	+	+	0	0	0	0	+	+	+	0	+	0	+	+	0	0	0	0	0	0	0	0	0	0	0	+	+	W+
10	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	0	0	+	+	+	+	0	+	+	0	+	0
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	+	0	+	0	+	0	+	+	W+
Patient cell																																	0

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

PEG Results (Crossmatch and Antibody Screen)

PEG crossmatch results		
	AHG	CC
Donor unit #1 (O-negative)	W+	NT
Donor unit #2 (O-negative)	0	2+

	AHG	CC
SC1	W+	NT
SC2	0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); W weak

Tube Panel

		Rh-hr								Kell				Duffy		Kidd		Lewis		MNS		P	Lutheran	Test results: IAT/tube PEG							
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC	
Cell #	Rh-hr																														
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	+	+	+	+	0	+	0	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	1+	NT	
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	0	+	0	0	+	0	+	+	0	0	0	0	0	+	+	+	0	+	1+	NT	
4	R ₀ r	+	0	0	+	+	0	+	0	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	+	0	+	W+	NT	
5	r' r	0	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	+	0	2+	
6	r'' r	0	0	+	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	0	+	0	2+	
7	rr	0	0	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	+	+	+	0	+	0	2+	
8	rr	0	0	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	0	+	0	0	+	+	+	+	0	+	0	2+	
9	rr	0	0	0	+	+	0	0	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	0	0	+	1+	NT		
10	rr	0	0	0	+	+	0	0	0	0	+	+	0	+	0	+	0	+	+	0	0	+	+	+	+	+	0	+	1+	NT	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	+	+	+	0	+	1+	NT	
Patient cell																		0	2+									0	2+		

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What antibodies did you identify?
2. What is the significance of the polyethylene glycol (PEG) panel in this case?
3. Why is there a difference between the reactions in the gel and PEG panels?
4. What may have occurred had the crossmatch been done using gel technique or had the weak reaction with PEG crossmatch for donor unit #1 been ignored?

Answers

1. **What antibodies did you identify?** Anti-Jk^a antibody is evident from the PEG panel, though only the homozygous cells are reactive (i.e., dosage effect; see Chap. 5, question 3 answer). In addition, note that the patient's RBC phenotype is Jk^a-negative/Jk^b-positive which helps to confirm the presence of alloanti-Jk^a antibody.
2. **What is the significance of the PEG panel in this case?** The PEG method was more sensitive toward detection of the Jk^a antibody in this patient; thus, the antibody could be identified on the PEG panel but not on the gel panel.
3. **Why is there a difference between the reactions in the gel and PEG panels?** For unknown reasons, sensitivity of antibody reactions may vary from one method to the next. In this case, PEG was more sensitive in detecting the anti-Jk^a than gel (also known as column agglutination). PEG and low ionic strength solution (LISS) are potentiating reagents that are added to increase the sensitivity of antibody detection via water exclusion (PEG) or alteration of the net surface charge (LISS) around RBCs. Gel and solid-phase red cell adherence methods are more recent technologies for immunohematology testing which can be automated. In gel testing, the reactants are centrifuged through a porous gel; in positive reactions (i.e., agglutination), the reactants are trapped inside the gel layers, while in a negative reaction, the reactants will settle to the bottom of the gel tube. In solid-phase testing, a positive reaction is indicated by diffuse adherence of reactants to the well, while in a negative reaction, the reactants settle in the center of the well (i.e., no adherence).
4. **What may have occurred had the crossmatch been done using gel technique or had the weak reaction with PEG crossmatch for donor unit #1 been ignored?** Under such circumstances, the anti-Jk^a would not have been identified, and the patient may have suffered from a hemolytic transfusion reaction due to incompatibility with donor unit #1.

Recommended Reading

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Chapter 13

The Transfusion Reaction



Clinical History

A 38-year-old man who underwent craniotomy surgery 2 months ago presents for outpatient red blood cell (RBC) transfusion of two units for symptomatic anemia and hemoglobin (Hgb) level of 7.4 g/dL. The patient had received multiple RBC transfusions during the craniotomy surgery and postoperatively in recovery; all of the RBC units were issued by immediate-spin (IS) crossmatch. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank along with a request for the two units of RBCs.

ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	4+	0	4+
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	0	0	2+	
SC2	0	0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

Two RBC units are issued by IS crossmatch and transfused without incident. The patient is discharged from the outpatient transfusion center. However, 3 days later, the patient returns to the emergency department because of a low-grade fever (temperature 100.3 °F) and weakness. The patient’s Hgb level in the emergency department is 8.0 g/dL. A posttransfusion sample (EDTA anticoagulant) is submitted to the blood bank for the workup of a suspected transfusion reaction.

Test Results: Posttransfusion Sample

<i>Clerical check</i>			
Patient: A-positive		Donor units	
		Unit #1	A-positive
		Unit #2	A-positive
<i>Visual check: no hemolysis</i>			
	Polyspecific	Anti-IgG	Anti-C ₃ d
DAT (pre-sample)	0	NT	NT
DAT (post-sample)	1+	1+	0

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Acid Eluate Panel (Posttransfusion Sample)

Cell #		Rh-hr	Rh-hr							Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube PEG				
			D	C	E	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M		N	S	s	P ₁	Lu ^a	Lu ^b	AHG
1		R ₁ wR ₁	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	0	2+
2		R ₁ R ₁	+	+	0	0	+	0	0	+	0	+	0	+	+	+	+	+	0	0	0	+	+	+	0	0	+	0	0	2+
3		R ₂ R ₂	+	0	+	0	0	0	0	0	+	0	+	0	0	+	+	+	0	0	0	0	+	+	+	+	+	0	0	2+
4		R ₀ r	+	0	0	+	+	0	+	0	0	+	0	+	0	0	0	+	0	0	0	0	+	+	+	+	0	0	0	2+
5		r'r	0	+	0	+	+	0	0	0	+	0	+	0	0	+	+	0	+	+	0	0	+	+	+	+	0	0	0	2+
6		r''r	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	0	+	+	+	+	0	0	0	2+
7		rr	0	0	0	+	+	0	0	+	0	+	0	+	0	+	+	+	0	0	0	0	+	+	+	+	0	0	0	2+
8		rr	0	0	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	0	+	+	+	+	+	0	0	0	2+
9		rr	0	0	0	+	+	0	0	0	+	+	0	+	0	+	+	0	+	0	0	+	+	+	0	0	0	0	0	2+
10		rr	0	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	+	+	+	0	0	0	2+
11		R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	+	+	+	0	0	0	2+
Last wash SC1																													0	2+
Last wash SC2																													0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Posttransfusion Sample Antibody Screen and Crossmatch

ABO/Rh (tube method)

Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	4+	0	4+

Antibody screen (tube LISS method)

	37 °C	AHG	CC
SC1	0	0	2+
SC2	0	0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Posttransfusion sample crossmatch

Donor unit	37 °C	AHG	CC
Unit #1	0	0	2+
Unit #2	0	1+	NT

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Questions

1. After reviewing the pre- and posttransfusion reaction sample type and antibody screen and the direct antiglobulin test (DAT) results, the results of the eluate panel, and the results of the posttransfusion sample crossmatches, what is your interpretation of the transfusion reaction?
2. How would you confirm your interpretation?
3. What is the significance of the IS crossmatches performed on the RBC units transfused to the patient?
4. How would you manage this patient's RBC transfusion needs in the future?

Answers

1. **After reviewing the pre- and posttransfusion reaction sample type and antibody screen and DAT results, the results of the eluate panel, and the results of the posttransfusion sample crossmatches, what is your interpretation of the transfusion reaction?** The patient in this case developed a fever of 100.3 °F 3 days after transfusion of RBCs in the outpatient department. Fever related to transfusion may be a nonspecific symptom of a transfusion reaction that may be secondary to acute or delayed reactions, immune or nonimmune reactions, or hemolytic or nonhemolytic reactions. Febrile nonhemolytic reaction (secondary to recipient leukoagglutinins or product cytokines) and septic reaction (due to bacterial contamination of the RBC product) may be considered but are usually

acute reactions and hence unlikely to be the cause of the transfusion reaction in this case. Transfusion-associated graft-versus-host disease causes a delayed reaction with fever but is characterized by pancytopenia, rash, and diarrhea. The posttransfusion DAT is immunoglobulin (Ig)G 1+ positive, suggestive of a delayed-type hemolytic transfusion reaction (i.e., extravascular hemolysis). The fact that the posttransfusion sample crossmatch with donor RBC unit #2 is 1+ incompatible in the face of a negative eluate panel suggests that the delayed-type hemolytic reaction is due to an antibody to a low-prevalence antigen present on donor unit #2 RBCs but not present on the RBCs of donor unit #1, on the screening cells, or on the panel cells. There are three principal situations in which the DAT result is positive for IgG but the eluate panel is negative: ABO incompatibility (as in ABO hemolytic disease of the fetus/newborn [HDFN]; see Chap. 11), presence of an antibody to a low-prevalence or rare antigen (as in this case), or in the case of a drug-induced autoantibody.

2. **How would you confirm your interpretation?** Considering that an antibody to a low-prevalence antigen is suspected in this case, it is unlikely that the specificity of the antibody will be determined by routine blood bank panel testing. Therefore, a posttransfusion sample should be sent to a specialized reference laboratory capable of determining antibody specificities to low- or high-prevalence antigens. Examples of antibodies to low-prevalence antigens that may be involved in this case include anti-Di^a (Diego blood group system, Di^a, found on band 3 protein of the RBC membrane, is present in less than 1% of Caucasians and African Americans but is a bit more prevalent among South American Indian, Chinese, and Japanese persons) and anti-Go^a (a low-prevalence antigen in the Rh blood group system associated with partial D antigen). Antibodies to both of these low-prevalence antigens are capable of causing hemolytic transfusion reactions as well as HDFN [1–3].
3. **What is the significance of the IS crossmatches performed on the RBC units transfused to the patient?** It is important to remember that IS crossmatch will only detect ABO incompatibility; thus, the alloantibody causing incompatibility to donor unit #2 in this case was not detected by the IS crossmatch. An antihuman globulin (AHG) crossmatch (often referred to as a full crossmatch) is necessary to detect this incompatibility (see Chap. 3 for more information about crossmatching).
4. **How would you manage this patient's RBC transfusion needs in the future?** As long as the antibody can be detected in the patient's serum or plasma, the patient may receive AHG crossmatch-compatible donor RBCs with little risk of hemolysis given that most donor RBCs will be compatible by virtue that they lack the low-prevalence antigen (although, the difficulty again would be that this would require rare antigen-positive cells for detection and likely would need to be accomplished in a reference laboratory). However, once the antibody becomes undetectable in the patient's serum or plasma, then it may be necessary to order antigen-negative RBCs (i.e., RBCs lacking Di^a or Go^a antigen) from a blood supplier in order to avoid the possibility of transfusing incompatible donor RBCs (i.e., Go^a antigen-positive RBCs). The latter is also true in case the patient develops a warm autoantibody, which would mask the presence of the alloantibody.

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Chapter 14

What’s This Junk?



Clinical History

A 53-year-old man, with history of coronary artery disease, hypertension, type 2 diabetes mellitus, and chronic anemia, presents with gangrenous left foot and is scheduled for amputation. The patient was transfused with red blood cells (RBCs) at the hospital 3 years ago and most recently 6 months ago; the antibody screen was negative at those times. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	0	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	1+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel and Enzyme Panel

		Rh-hr							Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results								
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel	Trypsin			
1	R ₁ wR ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	+	+	+	+	0	+	+	1+	0	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	+	+	0	0	
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	+	+	+	0	+	+	W+	0
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	+	+	0	+	+	1+	0
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	0
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	+	+	+	+	0	+	+	W+	0	
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	0	+	+	0	0	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	+	+	0	+	+	0	0	
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	+	0	0	+	+	0	0	+	+	0	+	+	W+	0
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+	+	+	0	+	+	W+	0	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	+	+	+	+	0	+	+	W+	0	
Patient cell																															0	NT	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Antibody Titration

Based on the weak and nonspecific reactions with the screening and panel cells as well as the results on the trypsin panel, the technologist working on the case suspected an antibody with the characteristic property of “high-titer/low-avidity” reactivity. Titration of the antibody was performed as given below:

Dilution									
	Undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
SC2	1+	1+	1+	1+	1+	1+	1+	W+	0

Reaction scale = 0 (no reaction) to 4+ (strong reaction); W weak

Questions

1. Does the serum titration result support or refute the technologist’s suspicion of a high-titer/low-avidity (HTLA) antibody?
2. Are HTLA antibodies clinically significant?
3. List at least three examples of HTLA antibodies.
4. How does the trypsin enzyme panel help you?

Answers

1. **Does the serum titration result support or refute the technologist’s suspicion of a HTLA antibody?** The serum titration result supports the presence of a HTLA antibody since there is a consistent weak (1+) reaction out to a titer of 64 (1:64 dilution). It should be noted that HTLA is not actually a category of antibodies, and the term is no longer in vogue, having been dropped by many serologists. Nevertheless, we use it here to describe an antibody that is characteristically weak and variably reacting with a relatively high titer.
2. **Are HTLA antibodies clinically significant?** Although HTLA antibodies are typically immunoglobulin (Ig)G class, they generally are not clinically significant since they do not cause hemolytic reactions or hemolytic disease of the fetus/newborn.
3. **List at least three examples of HTLA antibodies.** There are many antibodies that fall into the class of HTLA. Some examples are anti-Bg (Bennett–Goodspeed; human leukocyte antigen [HLA] remnants present on RBCs), anti-Ch (Chido) and anti-Rg (Rodgers; Ch and Rg antigens are located on C₄d which are adsorbed onto RBCs), anti-Cs^a (Cost collection), anti-JMH (John Milton Hagen system), anti-Kn^a (Knops system), anti-McC^a (McCoy and Knops system), and anti-Yk^a

(York and Knops system). In this case, the antibody was later confirmed to be anti-Kn^a based on testing in a specialized immunohematology reference laboratory using RBCs of the Knops null phenotype [1].

4. **How does the trypsin enzyme panel help you?** Reactivity of most, though not all, HTLA antibodies can be eliminated or reduced through enzyme treatment (especially trypsin and ficin) of the panel cells since the antigens are sensitive to such treatment. In this case, trypsin eliminated the reactivity of the antibody. The use of a reducing agent (which breaks disulfide bonds), such as dithiothreitol (DTT), can also weaken reactivity of many HTLA antibodies. Bg antigens (Bg^a and Bg^b) are an exception as they are resistant to ficin treatment but are sensitive to treatment with chloroquine and EDTA glycine acid (EGA). Knops antigens are also resistant to ficin and papain (though they are sensitive to trypsin and DTT). Although illustrated here for teaching purposes, note that trypsin and DTT reagents are not commonly used in hospital blood banks throughout the United States; however, antibody reference laboratories do make use of them.

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Chapter 15

Playing with Enzymes



Clinical History

A 70-year-old woman of African American descent, with history of hypertension, type 2 diabetes mellitus, anemia, and congestive heart failure, is admitted to the hospital because of symptomatic anemia (hemoglobin [Hgb] level, 7.2 g/dL). The patient has a history of red blood cell (RBC) transfusions and is known to have alloantibodies (anti-C, anti-K, and anti-Fy^a). The patient was last transfused two units of RBCs 2 weeks ago. Two RBC units are now requested, and a type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	2+			
SC2	2+			
Reaction scale = 0 (no reaction) to 4+ (strong reaction)				
Crossmatched RBCs (group O; C-, K-, Fy^a-antigen-negative)				
	AHG	CC		
Donor unit #1	1+	NT		
Donor unit #2	2+	NT		

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel and Enzyme Panel (Selected Cells)

		Rh-hr				Kell				Duffy		Kidd	Lewis		MNS				P	Lutheran		Test results										
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	J ^{s^a}	J ^{s^b}	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel	Ficin		
1	R ₂ R ₂	+	0	+	+	0	0	+	0	0	+	0	+	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	+	1+	1+
2	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0	0	+	0	0	+	0	+	1+	1+
3	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	+	+	+	0	+	0	+	0	0	0	+	0	+	+	+	+	1+	1+	1+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	+	+	0	0	+	+	+	0	0	0	+	0	+	+	0	+	0	0	0
5	R ₀ r	+	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	+	0	+	+	1+	1+
6	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	+	0	+	+	1+	1+
7	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	0	0	0	0	+	+	+	0	+	+	1+	1+
8	R ₀ r	+	0	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	+	+	0	+	+	+	+	+	+	0	+	+	2+	0
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	+	0	0	0	+	0	0	0	+	+	+	1+	1+
10	R ₀ r	+	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	+	+	+	+	0	+	1+	1+
Patient cell																														W+	NT	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

DAT profile		
Polyspecific:	W+	Anti-IgG: W+
		Anti-C ₃ d: 0

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Acid Eluate Panel

		Rh-hr							Kell			Duffy			Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube							
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC	Ficin	
1	R ₀ mR ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	+	+	+	+	0	+	1+	NT	0
2	RR ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	1+	NT	0	
3	RR ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	+	+	0	+	1+	NT	0
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	+	+	0	+	1+	NT	0
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	0	+	0	+	+	0	+	0	2+	NT
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	+	+	+	0	+	1+	NT	0
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	0	+	1+	NT	0
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	+	+	+	0	+	2+	NT	0
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	+	0	+	+	0	0	0	+	0	+	0	0	0	+	0	0	2+	NT
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+	+	+	0	+	1+	NT	0
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	0	+	+	0	+	2+	NT	0
Last wash SC1																														0	2+	NT
Last wash SC2																														0	2+	NT

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Antigen phenotype results (prior history)	
D+ C- E- c+ e+ K- Fy ^a - Fy ^b - Jk ^a + Jk ^b + S- s+ M+ N+ P ₁ + Le ^a - Le ^b +	

Selected-Cell (“Rule-Out”) Panel

		Rh-hr						Kell				Duffy		Kidd		Lewis		MNS		P		Test results: IAT tube LISS									
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	37 °C	AHG	CC
1	R ₀ R ₀	+	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	0	0	0	1+	NT
2	R ₂ r	+	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	0	0	1+	NT	
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	+	+	+	0	0	+	+	0	0	1+	NT	
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	+	0	+	+	+	+	+	0	0	0	2+	

Questions

1. Why are the two selected antigen-negative donor units incompatible?
2. What is the significance of the positive autocontrol and direct antiglobulin test (DAT) results?
3. Why are selected-cell (gel and “rule-out”) panels used in this case?
4. What do the antibody panels show? What is the significance of the Fy^a/Fy^b phenotype?
5. What is the significance of the Jk^a/Jk^b phenotype?
6. What is the significance of the panel results of the enzyme-treated cells?
7. In light of the findings, how would you manage this patient’s transfusion needs?

Answers

1. **Why are the two selected antigen-negative donor units incompatible?** The patient has developed one or more new alloantibodies.
2. **What is the significance of the positive autocontrol and DAT results?** The newly developed alloantibodies are coating donor RBCs transfused 2 weeks ago, causing the autocontrol and immunoglobulin (Ig)G DAT to be positive, possibly resulting in delayed (i.e., extravascular) hemolysis.
3. **Why are selected-cell (gel and “rule-out”) panels used in this case?** Panel cells are selected in this case to minimize positive reactions with antigens to which the patient is already known to be sensitized to by history (anti-C, anti-K) as well as with the newly identified anti-S (identified from the eluate panel). Thus, all four cells in the selected-cell panel are Rh(C)-, K-, and S-negative. However, the cells are selected to confirm reactivity with Fy^a and/or Fy^b, and the panel shows correlation between positive reactions and presence of either Fy^a or Fy^b antigen (in reality, the antibody is directed against a related antigen Fy3 discussed below in question 4 answer).
4. **What do the antibody panels show? What is the significance of the Fy^a/Fy^b phenotype?** The antibody panels show that the patient has developed anti-S antibody which is coating the RBCs as evidenced by the finding of anti-S antibody in the eluate panel. In addition, the gel and selected-cell (“rule-out”) panels show antibody with specificity to Fy^a and Fy^b antigens; since this antibody reactivity is not removed by enzyme treatment in the ficin panel (which is uncharacteristic of antibodies to either Fy^a or Fy^b; see table under question 6 answer below), and in light of the fact that the patient’s phenotype is negative for both Fy^a and Fy^b antigens, it is likely that the patient has anti-Fy3 antibody (which is not weakened by enzyme treatment). It should be noted that the Fy^a-negative-/Fy^b-negative-antigen phenotype has a high incidence in the African American population owing to resistance of this phenotype to the *Plasmodium vivax* malarial parasite. In this population, the most common reason for this phenotype is the presence of

the GATA-box promoter mutation that causes Fy^b antigen to be absent from red cell membranes but expressed on other tissues. Therefore, African American patients who are Fy^a - and Fy^b -negative by serologic typing do not make anti- Fy^b and only rarely make anti- $Fy3$ [1, 2].

5. **What is the significance of the Jk^a/Jk^b phenotype?** Antibodies to Jk^a and Jk^b cannot be ruled out on the gel panel since the nonreactive cell (cell #4) is heterozygous (i.e., Jk^a - and Jk^b -antigen-positive; antibodies to Jk^a and Jk^b should be ruled out only using homozygous cells; see Chap. 5, question 3 answer for discussion about dosage). Cell #8 (homozygous for Jk^b antigen) does provide some evidence, though, that anti- Jk^b is not present since the antibody reactivity is eliminated by ficin (Jk^a and Jk^b antigens are not enzyme-sensitive). Similarly, cell #4 on the selected cell (“rule-out”) panel cannot be used to eliminate antibodies to Jk^a and Jk^b because of heterozygous expression. However, since the patient is positive for both Jk^a and Jk^b antigens (phenotype results), the antibodies can be excluded.
6. **What is the significance of the panel results of the enzyme-treated cells?** Anti-S reactivity with ficin-treated cells is decreased, while anti- $Fy3$ reactivity is preserved. It is helpful to remember which antigens are resistant and which are sensitive to enzyme (ficin, papain, trypsin) treatment. Generally, a way to recall this is to use the names “Lewis P. Kidd, PhD” and “M.N.S. Duffy.” The former refers to the Lewis, P, Kidd, and Rh blood group system antigens which are resistant with increased reactivity to ficin or papain treatment, while the latter refers to the MNS and Duffy blood group system antigens which may be sensitive to ficin or papain (though, as noted, the $Fy3$ antigen is an exception). In addition, it is useful to know that the Kell blood group antigens are not affected by enzyme treatment but can be reduced by treatment with dithiothreitol (DTT), a reducing substance which cleaves disulfide bonds. The following table may also be a useful study aid for remembering the specific effect of each enzyme and DTT treatment on RBC antigens [3]:

Blood group system	Antigen	Enzyme/chemical		
		Ficin/papain	Trypsin	DTT
ABO	A, B	Resistant ↑	Resistant ↑	Resistant
Rh	D, C, c, E, e	Resistant ↑	Resistant ↑	Resistant
Kell	K, k	Resistant	Resistant	Sensitive
Lewis	Le^a , Le^b	Resistant ↑	Resistant ↑	Resistant
P	P_1	Resistant ↑	Resistant ↑	Resistant
MNS	M, N	Sensitive	Sensitive	Resistant
	S, s	Variable	Resistant	Resistant
	U	Resistant	Resistant	Resistant
Duffy	Fy^a , Fy^b	Sensitive	Resistant	Resistant
	$Fy3$	Resistant	Resistant	Resistant
Kidd	Jk^a , Jk^b	Resistant ↑	Resistant ↑	Resistant

Resistant ↑ = increased reactivity

7. **In light of the findings, how would you manage this patient's transfusion needs?** In addition to antigen-negative RBCs for previously identified alloantibodies (anti-C, anti-K, and anti-Fy^a), the patient also needs RBCs that are negative for S antigen and Fy3 antigen (RBCs lacking Fy3 antigen also lack Fy^a and Fy^b antigens). Although it is not known whether the previously identified anti-Fy^a is truly present in the patient's serum or whether the Fy^a specificity was just part of the now-apparent anti-Fy3, this is a moot point since the patient will be transfused with RBCs lacking Fy^a and Fy^b antigens (which, as noted, are negative for Fy3 antigen).

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Chapter 16

The Platelet Transfusion



Clinical History

A 55-year-old man with a history of alcohol abuse and liver cirrhosis presents to the emergency department with upper gastrointestinal bleeding and symptomatic anemia related to acute blood loss (hemoglobin [Hgb] level 7.1 g/dL). A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) along with an order for two units of red blood cells (RBCs) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	0	0	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

The patient, who has no prior history of transfusion, is transfused two units of A-negative RBCs by immediate-spin crossmatch. He is additionally transfused a unit of O-positive apheresis (single donor) platelets because of thrombocytopenia (platelets 30 K/ μ L). During transfusion of the platelet unit, the patient develops shaking chills, and the transfusion is discontinued after approximately three-quarters (200 mL) of the unit has been given (there is slight elevation of the temperature [T 99.8 $^{\circ}$ F], blood pressure remains unchanged at 130/70 mmHg, pulse slightly increased to 96/min from pretransfusion values). Posttransfusion samples are sent for transfusion reaction workup.

Test Results: Posttransfusion Sample

Clerical check			
Patient: A-negative		Donor unit	
		Apheresis platelet unit	O-positive
Visual check: no hemolysis			
	Polyspecific	Anti-IgG	Anti-C ₃ d
DAT (pre-sample)	0	0	0
DAT (post-sample)	2+	2+	0

Acid Eluate Panel

		Rh-hr										Kell				Duffy		Kidd	Lewis		MNS			P	Lutheran	Test results:IAT/tube PEG						
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC		
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	0	2+	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	0	0	+	0	2+	
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	0	+	+	0	0	0	0	+	+	+	+	0	0	0	2+	
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	+	0	0	0	2+	
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	0	0	0	+	0	+	+	0	0	0	2+	
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+	0	0	0	2+	
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	0	0	0	2+	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	0	+	+	+	+	0	0	0	0	2+	
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	0	0	0	0	0	0	2+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	+	+	+	0	0	0	0	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	0	0	0	0	2+
Last wash SC1																														0	2+	
Last wash SC2																														0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What are the possible causes of the transfusion reaction in this case? What is the most probable cause?
2. In consideration of the positive direct antiglobulin test (DAT) result in the post-transfusion sample, how do you interpret the results of the eluate panel?
3. What additional testing would you recommend to confirm the suspected cause of the transfusion reaction?
4. What precautions might have been taken to prevent this reaction?
5. Based on the Rh(D) type of the patient versus the apheresis product type, would you recommend Rh immune globulin (RhIg; 300 µg dose) to this patient? Would your recommendation change if this patient was a 25-year-old woman?

Answers

1. **What are the possible causes of the transfusion reaction in this case? What is the most probable cause?** The possibilities include immune hemolytic, febrile nonhemolytic (i.e., secondary to recipient leukocyte antibodies [antibodies to human leukocyte antigens, anti-HLA] or product cytokines), septic (i.e., bacterial contamination), and allergic reactions. In addition, the symptoms and signs exhibited (i.e., shaking chills and temperature elevation) may be unrelated to the platelet transfusion (i.e., may be secondary to the underlying disease state, reaction to medication, catheter sepsis, etc.). In this case, the most probable cause is a hemolytic reaction due to minor incompatibility from anti-A isoantibodies in the group-O apheresis platelet product (i.e., incompatible plasma).
2. **In consideration of the positive DAT result in the posttransfusion sample, how do you interpret the results of the eluate panel?** Although the DAT is immunoglobulin (Ig)G positive in the posttransfusion sample, the eluate panel is negative reflecting the fact that anti-A antibodies eluted from the patient's cells would not react with group-O panel cells. The three main reasons for a positive IgG DAT with a negative eluate result are listed in Chap. 13, question 1 answer.
3. **What additional testing would you recommend to confirm the suspected cause of the transfusion reaction?** Reacting the eluate against group-A₁ and group-B cells would show positive reaction only with the former and, thus, confirm hemolysis from donor anti-A as the cause of the reaction.
4. **What precautions might have been taken to prevent this reaction?** The reaction could have been prevented in several ways: (1) use of ABO-compatible apheresis platelets (however, this may not always be feasible since platelets have a short shelf life of only 5 days maximum and exclusive use of ABO-compatible platelets may lead to higher inventory requirements and wastage of platelet products), (2) processing of ABO-incompatible platelets to remove incompatible plasma by washing with saline (while technically achievable, it is a time-

consuming process in which the quality and number of platelets in the washed product is decreased; additionally, storage time due to concerns of bacterial contamination during the washing process, which is an open system, is limited to 4 h) or by use of platelet additive solution (PAS, this is a nutrient solution that replaces 70% of plasma in the platelet product) [1], or (3) measurement of anti-A₁ and anti-B isoantibody titers in the apheresis product and exclusion of such product if the antibody titer is high (i.e., anti-A₁ or anti-B titer greater than 100; while this method is relatively simple, it is not wholly reliable, and the cutoff for high isoantibody titer may be variable and arbitrary and may refer to either IgG or IgM titers). Finally, it is advisable to avoid giving platelets with ABO-incompatible plasma to small children or even adults of small stature/body weight where the amount of incompatible plasma given is relatively high compared to the patient's total whole-blood volume and would be expected to cause significant hemolysis.

5. **Based on the Rh(D) type of the patient versus the apheresis product type, would you recommend RhIg (300 µg dose) for this patient? Would your recommendation change if this patient was a 25-year-old woman?** RhIg may be given to an Rh-negative recipient of an Rh-positive platelet unit (either prior to or within 72 h after product administration) to prevent sensitization to the Rh(D) antigen, though such administration is far more critical in the case of a female of childbearing age, unlike the 55-year-old male patient in this case. While platelets themselves do not express Rh antigens, Rh antigens would be present and potentially immunizing on any RBC contaminants present in the platelet product (though usually not visible contamination; at least 2 mL of RBCs is necessary for visible contamination). In summary, many would not recommend RhIg administration to this older male patient but would certainly recommend it for the 25-year-old woman.

Reference

1. Van der Meer PF. PAS or plasma for storage of platelets? A concise review. *Transfus Med.* 2016;26:339–42.

Recommended Reading

- Cid J, Lozano M, Ziman A, West KA, et al. Low incidence of anti-D alloimmunization following D+ platelet transfusion. The anti-D alloimmunization after D-incompatible platelet transfusions (ADAPT) study. *Br J Haematol.* 2015;168(4):598–603.
- Klein HG, Anstee DJ. Haemolytic transfusion reactions. In: Klein HG, Anstee DJ, editors. *Mollison's blood transfusion in clinical medicine*. 12th ed. West Sussex: Wiley; 2014. p. 458–66.

Chapter 17

Differential Alloadsorption



Clinical History

A 62-year-old man is admitted with lower gastrointestinal bleeding and symptomatic anemia; his hemoglobin (Hgb) level is 5.0 g/dL. The patient has a known history of a warm autoantibody but no alloantibodies and was transfused two units of red blood cells (RBCs) 1 month ago at the hospital. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	4+	0	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	1+			
SC2	1+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel Panel

		Rh-hr										Kell				Duffy			Kidd		Lewis		MNS				P	Lutheran		Test results
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	J ^{s^a}	J ^{s^b}	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel	
1	R _W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	1+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	0	+	+	1+
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	+	0	0	0	0	+	0	+	+	0	+	1+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	+	0	+	1+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	+	0	0	+	0	+	+	0	+	1+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	0	0	+	0	0	+	+	+	+	+	0	+	1+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	0	+	0	+	+	+	0	+	1+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	0	+	+	0	+	1+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	0	+	+	0	0	+	0	+	0	0	0	+	+	1+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	+	+	+	0	+	1+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	+	0	+	1+
Patient cell																														1+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

DAT profile			
Polyspecific:	1+	Anti-IgG:	1+
		Anti-C ₃ d:	0

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Acid Eluate Panel

		Rh-hr				Kell				Duffy		Kidd		Lewis		MNS				P	Lutheran		Test results: IAT/tube PEG									
Cell #	Rh-hr	D	C	E	e	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC	
1	R ₁ W R ₁	+	+	0	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	2+	NT
2	R ₁ R ₁	+	+	0	0	+	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	0	+	2+	NT
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	+	+ ^S	0	+	2+	NT
4	R ₀ r	+	0	0	+	+	+	0	+	0	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	+	0	+	2+	NT
5	r' r	0	+	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	+	0	+	2+	NT
6	r'' r	0	0	+	+	+	+	0	0	0	0	+	0	+	0	+	+	+	0	0	0	+	+	+	+	+	+ ^w	0	+	2+	NT	
7	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	+	+	0	0	0	+	0	+	+	+ ^S	0	+	2+	NT
8	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	+	0	0	+	0	0	+	+	+	0	+	0	+	2+	NT
9	rr	0	0	0	+	+	+	0	0	0	0	+	+	+	0	+	0	+	+	0	0	0	+	0	+	0	0	0	+	+	2+	NT
10	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+	+ ^w	0	+	2+	NT	
11	R ₁ R ₁	+	+	0	0	+	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	0	+	2+	NT
Last wash SC1																															0	2+
Last wash SC2																															0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Differential Alloodsorption R₁R₁ Cells*

		Rh-hr						Kell						Duffy		Kidd		Lewis		MNS			P	Lutheran	Test results: IAT/tube PEG						
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC	
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	+	0	+	0	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	+	0	0	+	1+	NT
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	0	0	+	+	+	+	0	+	0	2+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	+	0	+	0	2+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	+	+	0	0	+	+	+	0	+	0	2+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	0	+	0	+	+	+	+	+	+	0	+	0	2+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	0	+	1+	NT
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	+	0	+	0	0	2+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	0	0	+	0	0	2+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	+	+	+	0	+	0	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	0	+	0	2+
Patient cell																													0	0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

*Patient's plasma adsorbed using ficin-treated R₁R₁ (K⁻, Jk^{a+}, Jk^{b-}) cells

Differential Alloadsorption R₂R₂ Cells*

Cell #	Rh-hr	Rh-hr						Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube PEG						
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a		Le ^b	M	N	S	s	Lu ^a	Lu ^b	AHG	CC
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	+	+	0	+	W+	NT
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	0	+	0	+	0	0	+	+	1+	NT
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	+	0	+	+	+	0	+	1+	NT
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	+	0	+	+	+	0	+	1+	NT
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	0	+	0	2+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	0	+	0	2+
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	0	+	W+	NT
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	+	0	+	0	2+	
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	0	0	+	0	0	+	0	0	+	+	1+	NT
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	+	0	+	1+	NT
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	+	0	+	1+	NT
Patient cell																													0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

*Patient's plasma adsorbed using ficin-treated R₂R₂ (K+, Jk^a-, Jk^b+) cells

Differential Alloodsorption rr Cells*

		Rh-hr							Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube PEG						
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC	
Cell #	Rh-hr																														
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	+	+	+	+	0	+	0	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	0	+	0	+	+	+	+	0	0	+	+	+	+	+	0	0	+	0	0	2+
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	+	+	0	0	0	2+
4	R ₀ r	+	0	0	+	+	+	0	+	0	0	+	0	+	0	0	0	0	+	0	0	0	+	+	+	+	0	0	+	0	2+
5	r' r	0	+	0	+	+	+	0	0	0	0	+	0	+	0	0	+	0	+	+	0	0	+	0	+	+	0	0	+	0	2+
6	r'' r	0	0	+	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	0	0	+	0	2+
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	0	0	+	0	2+
8	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	+	+	+	0	+	0	0	+	+	+	+	+	0	0	+	0	2+
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	+	0	+	0	0	+	0	+	0	0	0	0	+	0	2+
10	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	0	0	+	+	+	+	+	0	0	+	0	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	0	0	+	0	2+
Patient cell																														0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

*Patient's plasma adsorbed using ficin-treated rr (K⁺, Jk^{a+}, Jk^{b-}) cells

Questions

1. What antibodies did you identify?
2. Is the eluate panel of help in this case? Why or why not?
3. What is the principle of differential alloadsorption? Why is it used in this case as opposed to autoadsorption?
4. Why are the adsorbed cells treated with ficin? What antibody specificities cannot be identified using the ficin-treated cells?
5. What additional testing would you recommend to confirm the antibodies identified in the alloadsorption panels?

Answers

1. **What antibodies did you identify?** Warm autoantibody, known by history, is evident in the initial panel and in the eluate. Anti-K and anti-Jk^a are apparent in the differential alloadsorption panels.
2. **Is the eluate panel of help in this case? Why or why not?** The eluate panel is not helpful since it does not give any additional information in that the warm autoantibody is concentrated by the eluate and is masking identification of any alloantibody.
3. **What is the principle of differential alloadsorption? Why is it used in this case as opposed to autoadsorption?** Differential alloadsorption uses donor (allogeneic) group O RBCs to adsorb out autoantibodies. Since the phenotypes of the donor RBCs used to perform adsorption are different but known with respect to clinically significant antigens, such as Kell and Kidd, panels can be run to determine the presence of alloantibodies in the adsorbed serum or plasma. In this case, autoadsorption is not advised since the patient was recently transfused; autoadsorption should not be used in situations when there has been recent RBC transfusion (i.e., within the past 3 months) since alloantibodies could be adsorbed by donor RBCs. In addition, alloadsorption may be useful when the patient is severely anemic such that autoadsorption cannot be performed due to lack of patient's RBCs. A simpler variant of alloadsorption can be performed using phenotypically matched RBCs if the patient's antigen phenotype is known.
4. **Why are the adsorbed cells treated with ficin? What antibody specificities cannot be identified using the ficin-treated cells?** Ficin-treated RBCs are used to enhance adsorption of the warm autoantibodies, which often have specificity against Rh antigens. Since ficin destroys certain antigens, including Fy^a, Fy^b, M, N, S, and s, identification of antibodies to these antigens would be affected using ficin-treated cells (see Chap. 15, question 6 answer for table regarding enzyme affects on RBC antigens). It should be noted that alloadsorption can be performed not only using enzyme-treated cells but also using untreated and ZZAP-treated cells (ZZAP [1] reagent is a mixture of dithiothreitol [DTT] and papain).

In any case, one must know what antigens are present and intact on the adsorbing cells in order to interpret the panel results.

5. **What additional testing would you recommend to confirm the antibodies identified in the alloadsorption panels?** Since anti-K and anti-Jk^a are suspected from the adsorption panels, the patient would be expected to be K- and Jk^a-antigen negative. Thus, phenotyping the patient for these antigens is useful, if not serologically (which could be complicated by the autoantibody coating the patient's RBCs as well as recent RBC transfusion), then by molecular typing.

Reference

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Chapter 18

Hey, How Did That Antibody Get There?



Clinical History

A 33-year-old woman with a history of sickle cell disease is admitted with pain crisis (pain in extremities and back). The patient, who has no history of antibodies, has had several admissions to the hospital for pain crisis over the past 3 years and was transfused one unit of red blood cells (RBCs) 2 years ago; the RBCs were matched by extended phenotype (negative for C, E, and K antigens) as per your blood bank's policy for sickle cell patients. The patient denies receiving any RBC transfusions since the one unit given 2 years ago at the hospital. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	2+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel Panel

		Rh-hr										Kell				Duffy		Kidd		Lewis		MNS				P	Lutheran		Test results	
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel	
1	R _W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	0
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	0	+	+	2+
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	+	0	0	0	+	0	+	+	+	0	+	0
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	+	0	+	0
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	+	0	0	+	+	+	+	0	+	0
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	0	+	+	0	0	+	+	+	+	0	+	0
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	0	+	0	+	+	+	0	+	2+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	+	+	+	0	+	0
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	0	+	+	0	0	+	0	+	0	0	0	+	+	0
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	+	+	+	0	+	0
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	0	+	+	0	+	0
Patient cell																														2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

DAT profile		
Polyspecific: 2+		Anti-IgG: 2+
Reaction scale = 0 (no reaction) to 4+ (strong reaction)		Anti-C ₃ d: 0

Acid Eluate Panel

		Rh-hr							Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube PEG						
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC	
Cell #	Rh-hr																														
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	+	+	+	+	+	0	+	0	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	0	+	2+	NT	NT	2+
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	0	+	0	+	0	+	+	+	0	0	0	0	+	+	+	+	+	0	+	0	2+
4	R ₀ r	+	0	0	+	+	0	+	0	0	0	+	0	+	0	0	0	0	+	0	0	0	+	+	+	+	+	0	+	0	2+
5	r' r	0	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	0	+	0	0	+	+	+	+	+	0	+	0	2+
6	r'' r	0	0	+	+	+	0	0	0	0	0	+	0	+	+	+	+	0	+	0	+	+	+	+	+	+	+	0	+	0	2+
7	rr	0	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	+	0	+	0	2+
8	rr	0	0	0	+	+	0	0	0	0	0	+	0	+	+	+	+	+	+	0	0	+	+	+	+	+	+	0	+	0	2+
9	rr	0	0	0	+	+	0	0	0	0	+	+	0	+	0	+	+	+	0	0	+	+	0	+	0	0	0	0	+	0	2+
10	rr	0	0	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	0	+	+	+	+	+	+	+	0	+	0	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	+	0	+	0	2+
Last wash SC1																														0	2+
Last wash SC2																													0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What antibodies did you identify?
2. What is evident about the patient's transfusion history?
3. What is the principle of extended antigen-matched RBCs for sickle cell patients?

Answers

1. **What antibodies did you identify?** Anti-K antibody is present in the plasma and eluate.
2. **What is evident about the patient's transfusion history?** Although the patient denies RBC transfusion other than the one unit given 2 years ago, it is evident that the patient was transfused within the past 3 months. The evidence in this case is the positive direct antiglobulin test (DAT) with anti-K antibody in the eluate. Since the unit given 2 years ago was K-antigen negative (by extended antigen matching), the patient developed anti-K in response to a later transfusion given at another hospital which apparently does not employ extended antigen matching for sickle cell patients. One caveat, though, is that this may be an example of the Matuhasi–Ogata phenomenon, in which unexpected alloantibody is found in the eluate due to nonspecific immunoglobulin (Ig)G RBC uptake, though such nonspecific antibody binding usually occurs in association with specific binding of another alloantibody or even autoantibody (i.e., antibodies of one specificity might nonspecifically adhere to immune complexes of a different specificity) [1]. Finally, it should be noted that rare cases of autoanti-K mimicking an alloantibody have been reported [2]. This would likely be excluded in the case presented here if the patient was reliably known to be K-antigen negative by serologic typing, though, could be confirmed by molecular genotyping analysis if any doubt persisted.
3. **What is the principle of extended antigen-matched RBCs for sickle cell patients?** Sickle cell patients have a much higher rate of antibody development to red cell antigens after transfusion than non-sickle cell patients (20–50% vs. 2–8%) [3, 4]. The principle of extended antigen matching for sickle cell patient transfusion is that alloantibody development can be prevented by giving antigen-matched RBCs prior to primary sensitization. In many cases, blood banks will prophylactically match for the Rh group (C, c, E, e) and K antigens; in some cases, antigen matching will extend out to the Duffy and Kidd groups as well (particularly after the sickle cell patient has developed an alloantibody). There is no requirement or regulation, however, that blood banks must follow this protocol. Genotyping RBC antigens may also be useful for the purpose of extended antigen matching in sickle cell patients. As noted in Chap. 9, question 8, molecular-predictive red cell phenotyping is increasingly being used in clinical setting as an adjunct to classical immunohematology.

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Chapter 19

I Can't Stop the Hemolysis!



Clinical History

A 27-year-old woman with a history of sickle cell disease is admitted with pain crisis (pain in extremities and back) and possible sepsis. The patient, who has a history of anti-Fy^a and anti-S alloantibodies, has had several admissions to the hospital for pain crisis over the past 5 years. She was transfused two units of red blood cells (RBCs) 1 year ago and received RBC exchange transfusion (six units) for acute chest syndrome 2 years ago; all RBCs were matched by extended phenotype (C-, E-, K-negative) as per the blood bank's policy for sickle cell patients. A type and screen sample (ethylenediaminetetraacetic acid, EDTA anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	2+			
SC2	1+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel Panel

		Rh-hr										Kell				Duffy			Kidd		Lewis		MNS				P	Lutheran		Test results	
Cell #	Rh-hr	D	C	E	e	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel	
1	R _W R ₁	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	0
2	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	+	1+
3	R ₂ R ₂	+	0	+	0	+	0	0	0	0	0	+	0	+	0	+	0	0	+	0	0	0	0	+	0	+	+	+	0	+	0
4	R ₀ r	+	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	+	0	+	0
5	r'r	0	+	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	+	0	+	0
6	r''r	0	0	+	+	+	+	0	0	0	0	+	0	+	0	+	+	0	0	0	+	0	0	0	+	0	+	+	0	+	0
7	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	+	0	+	2+
8	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	+	0	+	0	0	+	0	+	+	+	0	+	0
9	rr	0	0	0	+	+	+	0	0	0	0	+	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	+	0	+	1+
10	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	+	0	0	0	0	0	+	0	0	0	0	+	+	0
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	+	0	+	+	0	+	0
Patient cell																															0

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Additional History

During the current admission, the patient was found to have a hemoglobin (Hgb) level of 6.4 g/dL and was transfused two units of RBCs (compatible by antihuman globulin [AHG] crossmatch, negative for Fy^a and S antigens). One day posttransfusion of the two RBC units, the Hgb was found to be 5.2 g/dL. An additional two RBC units were therefore requested and transfused (again AHG-crossmatch compatible, screened for Fy^a and S antigens). The following day, the Hgb was still not significantly improved (6.1 g/dL). In the meantime, the lactose dehydrogenase (LDH) level steadily increased from 300 to 1050 U/L. A posttransfusion antibody workup and direct antiglobulin test (DAT) profile are performed as follows.

DAT profile					
Polyspecific:	0	Anti-IgG:	NT	Anti-C ₃ d:	NT

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Posttransfusion Panel

		Rh-hr								Kell			Duffy			Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/ tube LISS								
		Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a		Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	37 °C	AHG	CC
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	0	+	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	0	0	0	2+	
2	R ₁ R ₁	+	+	0	0	+	0	+	0	0	0	+	0	+	0	+	+	+	+	0	0	+	0	+	0	+	0	0	+	0	1+	NT		
3	R ₂ R ₂	+	0	+	0	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	0	0	0	0	+	+	+	+	+	0	0	2+		
4	R ₀ r	+	0	0	+	+	+	0	+	0	0	+	0	+	0	+	+	0	0	+	0	0	0	0	+	+	+	+	+	0	0	2+		
5	r'r	0	+	0	+	+	+	0	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	0	+	+	+	+	0	0	2+		
6	r''r	0	0	+	+	+	+	0	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	0	+	+	+	+	0	2+	NT		
7	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	+	+	+	+	0	0	2+		
8	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	+	+	0	0	+	+	0	+	+	+	+	+	0	1+	NT		
9	rr	0	0	0	+	+	+	0	0	0	0	+	+	+	0	+	+	0	+	0	0	0	0	0	0	+	0	0	0	0	0	2+		
10	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	0	+	0	0	0	0	0	+	+	+	+	+	0	2+	NT		
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	+	0	+	0	0	0	0	0	+	0	+	+	+	0	0	0	2+	
Patient cell																															0	0	0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What antibodies are evident in the panels?
2. How do you explain the worsening anemia despite transfusion of crossmatch-compatible blood in this patient? How would you manage this patient's anemia?
3. Why is anti-S antibody not evident in the panels despite the patient's history of anti-S? Should this patient require additional RBC transfusion, is screening for S antigen-negative blood necessary given the panel findings?

Answers

1. **What antibodies are evident in the panels?** The panels show anti-Fy^a. Anti-S (known by history) is not showing in the panels.
2. **How do you explain the worsening anemia despite transfusion of crossmatch-compatible blood in this patient? How would you manage this patient's anemia?** Hyperhemolysis is a syndrome that is characterized by destruction of host cells and transfused donor cells by an unknown mechanism. It usually occurs in sickle cell patients though has been described in non-sickle cell patients as well. The patient in this case should be suspected of having hyperhemolysis since the hemoglobin level did not correct, and in fact was even lower, after receiving compatible blood transfusion. RBC transfusion, which may exacerbate the hemolysis, should be avoided in patients suspected of having hyperhemolysis whenever possible, and such patients should preferentially receive steroid or other treatments (such as intravenous immunoglobulin or rituximab [monoclonal anti-CD20/B-cell marker antibody]) to suppress the hemolysis [1–4]. A more novel treatment involves plasma-to-RBC exchange transfusion in which plasma is removed and replaced by RBCs [5]. The mechanism of hyperhemolysis is not well understood but may be related to destruction of RBCs via “bystander hemolysis” in which the RBCs are hemolyzed by antibodies without expressing the specific antigen or via “hyperactivated macrophages” [6]. Hyperhemolysis has also been classified into an acute form (occurring within 7 days, no new alloantibodies detected, and negative DAT) and a delayed form (occurring 7 days or later and associated with new alloantibodies and a positive DAT) [6, 7]; this case would fit into the acute form of hyperhemolysis.
3. **Why is anti-S antibody not evident in the panels despite the patient's history of anti-S? Should this patient require additional RBC transfusion, is screening for S antigen-negative blood necessary given the panel findings?** Anti-S is likely not showing up in the panel because the antibody titer has dropped below detectable levels (i.e., evanescence; see Chap. 5, question 2 answer). However, it is possible that the anti-S may have been misidentified to begin with; therefore, it is important to go back and review existing old antibody identification

panels as available (if the antibody was identified at your hospital). Phenotyping or genotyping the patient for the S antigen may also be useful, for if the patient is found to be S-antigen positive, then one may remove alloanti-S antibody from the patient's history profile. Based on current information though, the patient should continue to receive blood screened for S antigen in addition to Fy^a antigen until proven otherwise.

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Chapter 20

Just Another Autoantibody



Clinical History

A 21-year-old man is referred to the hospital’s emergency department (ED) by his primary care physician because of severe anemia (Hct 21%). The ED physician relates that the patient is a university student who has no significant past medical history and no history of transfusion. The patient was in his usual state of health until approximately 1 month ago when he experienced a flu-like illness following which he began to experience increasing fatigue and shortness of breath until he finally became quite lethargic and had difficulty to even get out of bed, prompting him to visit his doctor. A unit of RBCs is requested for transfusion. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	4+	4+	4+	0
<i>Antibody screen (AHG/gel method)</i>				
SC1	3+			
SC2	3+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel Panel

		Rh-hr										Kell				Duffy			Kidd		Lewis		MNS				P	Lutheran		Test results
Cell #	Rh-hr	D	C	E	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel		
1	R _W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	3+	
2	R ₁ R ₁	+	+	0	0	+	0	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	0	+	3+		
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	0	0	+	+	0	0	0	+	0	+	+	+	0	+	3+	
4	R ₀ r	+	0	0	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	+	0	+	3+	
5	r'r	0	+	0	+	+	0	0	0	0	+	0	+	0	0	0	+	0	+	+	0	0	+	+	+	+	0	+	3+	
6	r''r	0	0	+	+	+	0	0	0	0	+	0	+	0	+	0	0	0	+	0	0	+	+	+	+	+	0	+	3+	
7	rr	0	0	0	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	0	+	0	+	+	+	0	+	3+	
8	rr	0	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	+	0	+	3+	
9	rr	0	0	0	+	+	0	0	0	+	+	0	+	0	0	+	+	+	0	0	0	+	0	+	0	0	+	3+		
10	rr	0	0	0	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	+	+	+	0	+	3+	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	+	+	0	0	0	+	+	0	+	+	0	+	3+	
Patient cell																													3+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

DAT profile		
Polyspecific: 3+	Anti-IgG: 3+	Anti-C ₃ d: 3+
Reaction scale = 0 (no reaction) to 4+ (strong reaction)		

Tube Panel

Cell #	Rh-hr	Rh-hr							Kell				Duffy		Kidd	Lewis		MNS			P	Lutheran	Test results: IAT/tube LISS											
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a		Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b			Le ^a	Le ^b	M	N	S	s	P ₁	Lur ^a	Lur ^b	4 °C	37 °C	AHG
1	R _{1w} R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	0	2+	3+	NT	NT
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	+	+	0	0	+	+	+	+	0	+	0	2+	3+	NT	NT		
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	0	+	+	+	+	+	0	2+	3+	NT	NT		
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	0	+	+	+	+	+	0	2+	3+	NT	NT		
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	0	2+	3+	NT	NT		
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	0	2+	3+	NT	NT		
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	+	0	2+	3+	NT	NT		
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	+	+	0	2+	3+	NT	NT		
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	+	0	0	+	0	+	+	0	2+	3+	NT	NT		
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	0	2+	3+	NT	NT		
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	+	+	+	+	+	0	2+	3+	NT	NT		
Patient cell																												0	2+	3+	NT	NT		

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What antibodies are evident in the panels?
2. Can you rule out the presence of alloantibodies? Why or why not?
3. How would you manage this patient's transfusion needs?

Answers

1. **What antibodies are evident in the panels?** Strong warm autoantibody is present.
2. **Can you rule out the presence of alloantibodies? Why or why not?** The presence of alloantibodies cannot be ruled out from the panel; however, based on the fact that the patient is a male (i.e., cannot have a history of pregnancy) with no transfusion history, the presence of alloantibodies is unlikely. Therefore, although an autoadsorption test could be of use to uncover alloantibodies, in this case it is not necessary to do such testing given their unlikely presence.
3. **How would you manage this patient's transfusion needs?** Given the severe anemia, transfusion of least-incompatible RBCs may be considered to stabilize the patient. Steroids and other medical therapies (such as intravenous immunoglobulin [IVIG] or rituximab [monoclonal anti-CD20/B-cell marker antibody]) should also be considered. Splenectomy and therapeutic plasma exchange have also been used for treatment of severe warm autoimmune hemolytic anemia (WAIHA) (see also Chap. 7, question 5 answer). The term "least incompatible," strictly speaking, is one that many serologists do not like but is taken to mean that the crossmatch grade is not stronger than the autocontrol (i.e., in this case, a crossmatch not stronger than 3+). The idea behind this is that any crossmatch stronger than the autocontrol may be indicative of incompatibility due to the presence of alloantibodies in addition to the autoantibody. Not to be overlooked, the cause of the WAIHA should be investigated, and it is important to do a thorough workup of the patient to look for underlying causes of the autoantibody such as malignancy (particularly lymphoid malignancies), autoimmune disease, or infection. There have been some cases of WAIHA reported in association with infections, including influenza type A infection (noted since the patient in this case reported flu-like illness prior to the onset of WAIHA) [1] and parvovirus B19 infection [2], though infection (such as mycoplasma and Epstein-Barr virus) is more commonly found in association with cold agglutinin disease. Spontaneous remission of autoimmune hemolytic anemia (AIHA) weeks to months after resolution of acute infection may also occur, though a chronic AIHA course (lasting greater than 6 months) is more common in individuals less than 2 or older than 12 years of age [3].

References

1. Shizuma T. A patient with alcoholic liver cirrhosis who developed autoimmune hemolytic anemia following infection with influenza type A. *JSM Biotechnol Bioeng.* 2013;2(1):1019.
2. Giovannetti G, Pauselli S, Barrella G, Neri A, Antonetti L, Gentile G, et al. Severe warm autoimmune haemolytic anaemia due to anti-Jk^a autoantibody associated with parvovirus B19 infection in a child. *Blood Transfus.* 2013;11(4):634–5.
3. Bercovitz RS, Macy M, Ambruso DR. A case of autoimmune hemolytic anemia with anti-D specificity in a one-year-old. *Immunohematology/American Red Cross.* 2013;29(1):15–8.

Recommended Reading

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Chapter 21

I Got You Baby



Clinical History

A 31-year-old woman is post uncomplicated vaginal delivery of a full-term, healthy baby 1 day ago. According to the history from the patient's obstetrician, the patient's blood type is O-negative, as tested from an outside laboratory, and she received Rh immunoglobulin (RhIg) at 28 weeks of gestation. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) from the mother and cord blood is submitted to the blood bank along with a request for postpartum RhIg.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	1+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	1+			
SC2	2+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Test Results: Cord Blood

ABO/Rh (tube method)			
Baby's RBCs (forward typing)			
Anti-A	Anti-B	Anti-D	Weak D
0	0	4+	NT
Cord anti-IgG DAT: 0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel Panel

		Rh-hr						Kell						Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results				
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	J ^S	J ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel	
1	R _{1w} R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	+	1+
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	0	+	0	+	^S	0	+	+	2+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	+	+	0	+	1+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	0	+	+	+	^w	0	+	0
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+	0	+	0
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	+	+	^S	0	+	+	0
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	0	^S	0	+	+	0
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	+	0	0	+	0	+	0	0	0	+	0
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	^w	0	+	+	0
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	+	+	+	0	+	1+
Patient cell																														0

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Antibody titration	
Rh(D) antibody titer: 2	

Questions

1. What is the Rh(D) type of the patient?
2. What is the significance of the cord direct antiglobulin test (DAT) result?
3. What is the significance of the Rh(D) antibody titration result?
4. Do you advise that the patient receive postpartum RhIg?
5. Would a fetal screen (rosette) test be of use in screening for fetal–maternal hemorrhage (FMH) in this case? If not, how would you advise to screen for FMH in this case?

Answers

1. **What is the Rh(D) type of the patient?** There is a discrepancy between the reported Rh(D) typing (Rh-negative) from the outside laboratory and the Rh(D) result from the hospital blood bank (Rh-positive). On closer inspection, however, it is apparent that the Rh(D)-typing result from the blood bank is weak (i.e., only a 1+ result). Because testing laboratories use different monoclonal D-typing reagents and testing methodologies (i.e., tube vs. gel testing, etc.), the Rh(D)-typing result can vary in the case that a patient has a variant of the Rh(D) antigen such as a weak or partial D expression.
2. **What is the significance of the cord DAT result?** The panel shows a clear anti-D antibody which may cause HDFN if it crossed the placental barrier and coated the baby's red blood cells (RBCs), since the baby is Rh(D) positive. The cord immunoglobulin (Ig)G DAT is negative, however. Given the maternal history of prenatal RhIg as well as the low antibody titer of the anti-D (titer of 2), it is likely that the source of the antibody is from the RhIg. Anti-D passively acquired from RhIg has a very low risk to the baby and does not cause HDFN itself. Of interest, though the mother's RBCs express Rh(D) antigen, the autocontrol is negative on the panel, likely because of the weak D expression. Also note that RhIg does not affect weak D typing.
3. **What is the significance of the Rh(D) antibody titration result?** Antibody titration is not routinely performed in cases in which presence of anti-D antibody is highly suspected to be a result of passive RhIg immunization based on history and characteristic weak strength of the anti-D reactions on the antibody panel. In this case, however, some of the anti-D reactions on the antibody screen and the identification panel are reacting at moderate strength (2+), and so an antibody titration is performed. The titer is 2, though, which is consistent with passively acquired anti-D from RhIg.
4. **Do you advise that the patient receive postpartum RhIg?** As noted above, the mother appears to be a D variant, likely a weak D type (formerly known as D^U). While there are a number of weak D subclassifications, suffice it to say that the majority of people who type as weak D (types 1–3 are most common, particularly

in Caucasians) are not at risk of becoming sensitized (i.e., they do not produce immune anti-D upon exposure to D antigen), and, therefore, they do not require RhIg [1, 2]. However, some partial D types (formerly referred to as D mosaic), of which there are a multitude of subclassifications, may become sensitized and, thus, do require RhIg prophylaxis. Those with partial DVI type are at particular risk so that Rh(D)-typing reagents are designed to type partial DVI as Rh-negative. In such situations, one may consider testing the patient's RBCs using a partial D-typing kit or sending the patient's sample for genetic analysis to sort out the cause of the D-variant result or discrepancy in the Rh(D) typing; this would determine the patient's risk of becoming sensitized to the Rh(D) antigen and whether RhIg prophylaxis is necessary [3]. The AABB-CAP Interorganizational Work Group on RHD Genotyping recommends that Rh(D) genotyping should be performed whenever a serologic weak D phenotype is detected in a patient, especially in pregnant women or women of child-bearing age [4].

5. **Would a fetal screen (rosette) test be of use in screening for FMH in this case? If not, how would you advise to screen for fetal–maternal hemorrhage in this case?** The fetal screen test is used to determine the presence of excess FMH such that more than a single dose of RhIg is necessary to prevent Rh(D) sensitization. The principle of the test is that it uses the maternal Rh-negative blood sample to screen for the presence of fetal Rh-positive RBCs. The test, however, is merely qualitative, and if positive, a quantitative test (such as the Kleihauer–Betke [KB] test or flow cytometry) must be performed to determine the extent of the FMH. In this case, because the mother's RBCs are typing as Rh(D)-positive (even if weak D), the fetal screen test cannot be used because the sample will test strongly positive (i.e., falsely indicating excess FMH). Thus, only KB or flow cytometry testing (which alternatively identifies fetal RBCs through the presence of fetal hemoglobin) can be used in this situation to test for FMH if necessary.

References

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3. Sandler SG, Flegel WA, Westhoff CM, et al. It's time to phase in RHD genotyping for patients with a serologic weak D phenotype. *Transfusion*. 2015;55(3):680–9.
4. Haspel R, Westhoff CM. How do I manage Rh typing in obstetrics patients? *Transfusion*. 2015;55(3):470–4.

Chapter 22

“You” Got that Right



Clinical History

A 63-year-old African American man with a history of hypertension is admitted to the hospital with sepsis and hypotension and is scheduled for a lumbar puncture procedure. The hemoglobin (Hgb) level is 6.9 g/dL, the platelet count is 65 K/ μ L, the prothrombin time (PT) is 16.5 s (international normalized ratio [INR] 1.4), and the activated partial thromboplastin time (aPTT) is 42.0 s. The patient’s transfusion history is unknown. There is a request for two units of red blood cells (RBCs), one unit of single donor (apheresis) platelets, and one unit of fresh frozen plasma (FFP) prior to the lumbar puncture. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	2+			
SC2	2+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel Panel

		Rh-hr								Kell				Duffy		Kidd		Lewis		MNS				P	Lutheran		Test results				
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel		
1	R ₁ W ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	2+	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	0	0	0	0	+	+	0	
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	+	+	0	0	0	+	0	+	+	^S	0	+	2+	
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	+	0	+	2+	
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	0	0	+	0	+	+	0	+	2+	
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	0	+	0	0	+	+	+	^w	0	+	+	2+	
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+	^S	0	+	2+	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	0	0	^S	0	+	+	0	
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	0	0	+	+	2+	
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	+	^w	0	+	+	2+	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	0	+	+	0	+	+	2+
Patient cell																														0	

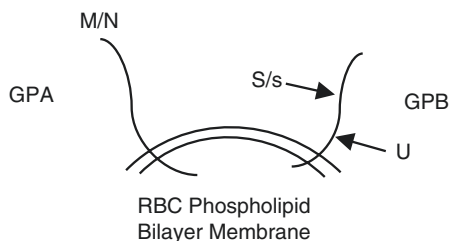
Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. Based on the panel, what antibody do you suspect is present in the patient's plasma? Is the antibody clinically significant?
2. What is the significance of the patient's S and s antigen phenotype? What structure is the patient's RBCs lacking?
3. How would you manage this patient's RBC transfusion needs at this time?
4. What is your recommendation for platelet and plasma transfusion in the patient at this time?

Answers

1. **Based on the panel, what antibody do you suspect is present in the patient's plasma? Is the antibody clinically significant?** An antibody to a high-frequency antigen is likely present; an autoantibody is excluded based on the negative auto-control. Given that the only nonreactive cells on the panel are negative for both the S and s antigens (a rather uncommon phenotype), one would suspect an antibody is present to the U antigen. Further suspicion is aroused by the fact that the patient's phenotype is also negative for the S and s antigens and that the patient is African American whereby the rare S-s-U-negative phenotype is more prevalent, though even so, the frequency is still below 1%. Anti-U is a clinically-significant immunoglobulin (Ig)G, warm-reacting antibody capable of causing delayed hemolysis and hemolytic disease of the fetus/newborn (HDFN).
2. **What is the significance of the patient's S and s antigen phenotype? What structure is the patient's RBCs lacking?** The M and N antigens are located on glycophorin A (GPA), while the S, s, and U antigens are located on glycophorin B (GPB). Given the patient's phenotype (S- and s-antigen negative), the patient lacks glycophorin B and, thus, is also U-antigen negative. The diagram below will help you to visualize the GPA and GPB structures on the RBC membrane.



3. **How would you manage this patient's RBC transfusion needs at this time?** Rare U-antigen-negative blood will be needed for this patient; such RBC units

usually must be obtained as frozen-thawed (deglycerolized) RBCs requiring special handling due to their shortened shelf life (24 h after thawing).

4. **What is your recommendation for platelet and plasma transfusion in the patient at this time?** Regarding platelet transfusion, though guidelines typically recommend platelets to be at least 100 K/ μ L for neurosurgical procedures, this does not necessarily apply to lumbar puncture whereby many consider 50 K/ μ L to be adequate [1]. Therefore, given this patient's platelet count of 65 K/ μ L, platelet transfusion is not recommended prior to the lumbar puncture. Similarly, because the PT (16.5 s) and aPTT (42.0 s) are only minimally elevated in this patient, FFP is not recommended given that the patient has adequate levels (i.e., greater than 50% of normal) of essential coagulation factors for surgical hemostasis. In addition, transfusion of a single unit of plasma as requested in this patient would not be a sufficient dose (generally, the recommended volume of plasma transfusion is 10–15 mL/kg so that multiple units are necessary; each unit of FFP ranges in volume from 200 to 350 mL of plasma).

Reference

1. Kaufman RM, Djulbegovic B, Gernsheimer T, Kleinman S, Tinmouth AT, Capocelli KE, et al. Platelet transfusion: a clinical practice guideline from the AABB. *Ann Intern Med.* 2015;162(3):205–13.

Recommended Reading

- Kaufman RM, Djulbegovic B, Gernsheimer T, et al. Platelet transfusion: a clinical practice guideline from the AABB. *Ann Intern Med.* 2015;162(3):205–13.
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Chapter 23

The Case of Low Platelets



Clinical History

A 30-year-old woman presents for outpatient transfusion of one unit of red blood cells (RBCs). The patient's hemoglobin (Hgb) level is 7.5 g/dL, and the platelet count is 25 K/ μ L (the patient is known to have a history of thrombocytopenia). The patient has no history of transfusion or pregnancy. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	4+	0	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	2+			
SC2	2+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel Panel

Cell #	Rh-hr	Rh-hr										Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	Lu ^a	Lu ^b	
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	0	+	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	+	2+
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	0	+	+	0	+	2+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	+	+	+	+	0	+	2+
5	r' r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	0	+	0	0	+	+	+	0	+	0
6	r'' r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+ ^w	0	+	0
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+ ^s	0	+	0
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+ ^s	0	+	0
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	+	0	+	0	0	0	+	0
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	0	+	0
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	0	+	0	0	+	2+
Patient cell																												2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

DAT profile		Anti-IgG: 2+		Anti-C ₃ d: 0	
Polyspecific: 2+					

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Acid Eluate Panel

		Rh-hr							Kell				Duffy		Kidd	Lewis		MNS			P	Lutheran	Test results:										
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a		Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b			Le ^a	Le ^b	M	N	S	s	P1	Lr ^a	Lu ^b	IAT/tube	PEG
Cell #	Rh-hr	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	0	+	+	2+	NT	NT
1	R ₁ R ₁																																
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	2+	NT	NT
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	0	+	+	+	+	+	0	+	2+	NT	NT	NT
4	R ₀ r	+	0	0	+	+	+	0	+	0	0	+	0	+	0	0	0	0	0	0	0	0	+	+	+	+	+	0	+	2+	NT	NT	NT
5	r'r	0	+	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	+	+	0	+	0	2+	2+	2+
6	r''r	0	0	+	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	0	2+	2+	2+
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	0	+	0	2+	2+	2+
8	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	+	0	+	0	2+	2+	2+
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	0	0	0	+	0	+	0	0	0	+	0	0	2+	2+	2+
10	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	0	0	0	+	+	+	+	+	+	+	0	+	0	2+	2+	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	+	+	+	0	+	2+	NT	NT	NT
Last wash SC1																															0	2+	2+
Last wash SC2																															0	2+	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What antibodies did you identify in the patient's sample?
2. What are the possible sources of the antibodies, and how does the patient's history and laboratory values lead you to the most likely source?
3. In consideration of the platelet count, is platelet transfusion indicated for this patient? Why or why not?
4. In light of the patient's Rh(D) type and the antibody present in the panel, would you transfuse this patient with Rh-positive or Rh-negative RBCs?

Answers

1. **What antibodies did you identify in the patient's sample?** Anti-D is apparently present. However, given that the patient is Rh-positive, it is not certain from the panel whether the antibody is an auto- or an alloantibody. The latter would occur in the case of a partial D type (see Chap. 21, question 4 answer, for more information on partial D). Adsorption with Rh(D)-positive and Rh(D)-negative cells could possibly differentiate the two as an autoanti-D (which has broad reactivity) could be removed by adsorption with both Rh(D)-positive and Rh(D)-negative cells, while an alloanti-D could be removed only by adsorption with Rh(D)-positive cells. Finally, an autoanti-LW (LW is a blood group antigen that is closely associated with the D antigen) should also be considered and may be distinguished through testing with dithiothreitol (DTT)-treated cells; DTT destroys LW antigen but not Rh(D) antigen.
2. **What are the possible sources of the antibodies, and how does the patient's history and laboratory values lead you to the most likely source?** In light of the above discussion, alloanti-D may have been acquired through prior transfusion or pregnancy exposure to the Rh(D) antigen if the patient is a partial D type. However, in this case, the patient does not have a history of transfusion or pregnancy, and because of the patient's thrombocytopenia, the patient was suspected to have idiopathic (immune) thrombocytopenic purpura (ITP), and history was promptly obtained that in fact, the patient had received intravenous Rh immunoglobulin (IV RhIg) several weeks ago for treatment of the ITP. Thus, based upon this history, it is apparent that the anti-D was passively acquired, and further workup to differentiate an autoanti-D or an autoanti-LW was unnecessary. IV RhIg is specifically indicated for treatment of ITP in patients who are Rh-positive, who have a functioning spleen, and who are not significantly anemic (since the treatment causes further anemia through immunoglobulin [Ig]G coating of Rh-positive RBCs and removal via the spleen, essentially an iatrogenic hemolytic anemia). For more information on IV RhIg in the treatment of thrombocytopenia, refer to Chap. 7, question 6.

3. **Is platelet transfusion indicated for this patient? Why or why not?** Since the patient has ITP, platelet transfusion is generally contraindicated unless there is life-threatening bleeding.
4. **In light of the patient's Rh(D) type and the antibody present in the panel, would you transfuse this patient with Rh-positive or Rh-negative RBCs?** Since the goal of RBC transfusion is to treat the patient's anemia (i.e., increase the Hgb level), Rh-negative RBCs might be paradoxically given to this Rh-positive patient in the presence of the passively acquired anti-D (which could shorten the survival of transfused Rh-positive RBCs).

Recommended Reading

1. Klein HG, Anstee DJ. The transfusion of platelets, leucocytes, haematopoietic progenitor cells and plasma components. In: Klein HG, Anstee DJ, editors. *Mollison's blood transfusion in clinical medicine*. 12th ed. West Sussex: Wiley; 2014. p. 625.

Chapter 24

The Perils of Transfusing the Sickle Cell Patient



Clinical History

A 44-year-old woman with sickle cell disease presents to the emergency department (ED) with acute pain crisis (pain in shoulders and upper back). The patient's hemoglobin (Hgb) level is 7.2 g/dL, which is around her usual baseline Hgb. The patient received two units of red blood cells (RBCs) in the outpatient department of another hospital 4 days prior to admission (her Hgb level was 7.6 g/dL prior to transfusion at that time), and as per that hospital's blood bank, the patient has an antibody history of warm autoantibody, anti-Fy^a, and anti-Jk^b. Overnight in the emergency department, the patient's blood pressure dropped to 90/56 mmHg, and the Hgb level dropped to 5.4 g/dL with an elevated total bilirubin level of 15.3 mg/dL and lactate dehydrogenase (LDH) level of 10,000 U/L. The patient is admitted to the medical intensive care unit (MICU), and a type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank along with a request for two units of RBCs.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	1+			
SC2	1+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel Panel

Cell #	Rh-hr	Rh-hr								Kell				Duffy		Kidd		Lewis		MNS				P	Lutheran		Test results			
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N		s	s		P ₁	Lu ^a	Lu ^b
1	R _W R ₁	+	+	0	0	+	0	+	0	0	+	+	0	+	0	0	+	+	+	0	+	0	+	+	+	+	0	+		W+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	+	0	0	+		W+
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	0	+	0	+	0	0	+	+	0	0	0	0	+	0	+	+	0	+		W+
4	R ₀ r	+	0	0	+	+	+	0	+	0	0	+	0	+	0	0	0	+	0	0	0	0	+	+	+	+	0	+		1+
5	r'r	0	+	0	+	+	+	0	0	0	0	+	0	+	0	0	+	+	0	+	0	0	0	0	+	+	0	+		W+
6	r''r	0	0	+	+	+	+	0	0	0	0	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+	0	+		1+
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	0	+	+	+	0	0	0	+	0	+	+	0	+		W+
8	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	+	+	+	0	+	+	0	0	+	+	+	+	0	+		W+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	+	0	0	+	0	0	0	+		W+
10	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	+	0	+	+	0	0	+	+	+	+	+	+	0	+		1+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	0	+	0	+	0	0	+	+	0	0	0	+	+	0	0	+	0	+		W+
Patient cell																														W+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

DAT profile		
Polyspecific: W+	Anti-IgG: W+	Anti-C ₃ d: 0

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Acid Eluate Panel

Cell #		Rh-hr								Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/ tube PEG					
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC	
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	W+	NT	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	+	0	0	0	+	+	+	0	0	+	W+	NT		
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	+	0	+	+	+	0	+	W+	NT	
4	R ₀ r	+	0	0	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	+	0	+	W+	NT	
5	r' r	0	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	W+	NT	
6	r'' r	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	W+	NT	
7	rr	0	0	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	0	+	W+	NT	
8	rr	0	0	0	+	+	0	0	0	0	+	0	+	0	+	+	+	0	+	0	0	+	0	+	+	+	0	+	W+	NT	
9	rr	0	0	0	+	+	0	0	0	0	+	+	0	+	0	+	+	0	0	0	+	0	+	0	0	0	+	0	+	W+	NT
10	rr	0	0	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	0	0	+	+	+	+	+	+	0	+	W+	NT	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	W+	NT	
Last wash SC1																													0	2+	
Last wash SC2																													0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong); S strong, W weak

Tube and Enzyme Panel

		Rh-hr										Kell			Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/ tube				
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC	Ficin
Cell #	Rh-hr	+	+	0	0	+	0	+	0	0	0	+	0	+	0	0	+	+	+	0	+	0	+	+	+	+	+	0	2+	NT	
1	R ₁ R ₁																														
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	+	+	0	0	0	+	+	+	0	0	+	0	2+	NT	
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	0	+	0	+	0	0	+	+	0	0	0	0	+	+	+	+	+	0	2+	NT	
4	R ₀ r	+	0	0	+	+	+	0	+	0	0	+	0	+	0	0	0	0	0	0	0	0	+	+	+	+	0	NT	W+	W+	
5	r'r	0	+	0	+	+	+	0	0	0	0	+	0	+	0	0	+	+	0	+	+	0	0	+	+	+	0	2+	NT		
6	r''r	0	0	+	+	+	+	0	0	0	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	+	0	NT	0	0	
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	0	+	+	+	0	0	0	+	+	+	+	0	2+	NT		
8	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	+	0	2+	NT		
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	0	+	+	0	+	0	0	+	+	+	0	2+	NT			
10	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	0	0	0	0	+	+	+	+	+	0	NT	0	0	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	+	+	0	0	0	+	+	+	+	+	0	2+	NT		
Patient cell																											0	2+	NT		

Reaction scale = 0 (no reaction) to 4+ (strong); S strong, W weak

Selected-Cell (“Rule-Out”) Panel

		Rh-hr							Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/ tube PEG							
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC		
1	R ₀ r	+	0	0	+	+	+	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	+	+	+	0	0	+	+	0	W+	NT
2	R ₀ r	+	0	0	+	+	+	0	0	0	0	0	+	0	+	0	0	0	+	0	+	0	+	+	+	0	0	+	+	0	0	2+
3	R ₀ r	+	0	0	+	+	+	0	+	+	+	0	+	0	+	0	0	0	+	0	+	0	+	0	0	+	0	+	+	0	W+	NT
4	R ₀ r	+	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	0	+	0	+	0	+	0	0	0	0	+	+	0	0	2+
5	R ₀ r	+	0	0	+	+	+	0	0	0	0	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+	+	0	W+	NT	

Reaction scale = 0 (no reaction) to 4+ (strong reaction) ; S strong, W weak

Additional History

The patient is transfused two units of antigen-compatible RBCs, based on antibody history and the above antibody workup, and the Hgb level rises to 7.5 g/dL and the patient's vitals stabilize. However, the following morning, the Hgb level drops to 5.2 g/dL, and the LDH level has risen to 14,000 U/L. The patient is treated with steroids, intravenous immunoglobulin (IVIG), and further transfusions of RBCs as well as plasma, platelets, and cryoprecipitate for disseminated intravascular coagulation (DIC). Sodium bicarbonate is administered for lactic acidosis and vasopressor medication (norepinephrine) is given to maintain blood pressure. Nevertheless, multiorgan failure follows, and the patient goes into cardiopulmonary arrest and cannot be resuscitated.

Questions

1. What antibodies did you identify in the patient's sample?
2. Could you identify all antibodies in the patient's sample according to the patient's antibody history? If not, can you explain why one or more antibodies are not identifiable in the panels?
3. Can you explain what may have led to the sudden demise of this patient?
4. In consideration of the transfusion-related death of the patient, what reporting requirements does the transfusion service have?

Answers

1. **What antibodies did you identify in the patient's sample?** From the panels, one can identify anti-Fy^a (reactive with homozygous cells only) and an apparent new anti-V alloantibody. In addition, the weak warm autoantibody, as indicated by history, is reactive in the patient's gel panel and in the eluate.
2. **Could you identify all antibodies in the patient's sample according to the patient's antibody history? If not, can you explain why one or more antibodies are not identifiable in the panels?** As indicated by history, the patient has an anti-Jk^b which is not present in the panels. Antibodies to Kidd blood group antigens (i.e., anti-Jk^a and anti-Jk^b) notoriously disappear over time; that is, the antibody titers drop to undetectable levels (i.e., evanescence; see Chap. 5, question 2 answer) placing the patient at great risk of a hemolytic reaction if the history of the antibody is unknown and the patient unwittingly gets re-exposed to the offending Kidd antigen. In such a case, the patient may experience brisk hemolysis since anti-Jk^a and anti-Jk^b may fix complement.

3. Can you explain what may have led to the sudden demise of this patient?

Putting the whole picture together, it appears that the patient developed a previously unidentified anti-V antibody which likely reacted with one or both units given 4 days prior to the ED visit, triggering a delayed hemolytic reaction and sickle crisis. This appears to be further complicated by hyperhemolysis (see Chap. 19, question 2 answer), exacerbated by the two additional antigen-negative (i.e., Fy^a-, Jk^b- and V-negative) RBC units that were subsequently transfused. Though the V antigen is a low-frequency antigen (belonging to the Rh blood group system) among Caucasians (about 1%), it has a higher frequency among African Americans (about 30%). Since many hospital transfusion services attempt to prevent alloimmunization in transfused sickle cell patients via the strategy of extended phenotype matching (see Chap. 18, question 3 answer), it is likely that this patient received blood from donors with higher V-antigen frequency.

4. In consideration of the transfusion-related death of the patient, what reporting requirements does the transfusion service have? The transfusion service medical director must immediately report the fatality to the Centers for Biologics Evaluation and Research (CBER) of the US Food and Drug Administration (FDA) by telephone and submit a written report within 7 days. Additional reporting to CBER is required for any follow-up information obtained beyond the 7-day period (e.g., an autopsy report). Besides the FDA, other regional or local authorities, such as state or city departments of health, may also have reporting requirements in the case of a transfusion-related fatality. Finally, the transfusion service may voluntarily participate in and report to the National Healthcare Safety Network (NHSN) Hemovigilance Module (of note, though, outside of the United States, such hemovigilance reporting is mandatory in France where the concept started back in the early 1990s).

Recommended Reading

Win N, Doughty H, Telfer P, Wild BJ, Pearson TC. Hyperhemolytic transfusion reaction in sickle cell disease. *Transfusion*. 2001;41(3):323–8.

Klein HG, Anstee DJ. Haemolytic transfusion reactions. In: Klein HG, Anstee DJ, editors. *Mollison's blood transfusion in clinical medicine*. 12th ed. West Sussex: Wiley; 2014. p. 472.

Chapter 25

To KB or Not to KB, That Is the Question



Clinical History

A 36-year-old woman, with no significant past medical history, is *status post* vaginal delivery of a baby at term pregnancy. The postdelivery hematocrit (Hct) is 30%, and the patient weighs 65 kg. The patient is known to have received prenatal Rh immunoglobulin (RhIg) at 28 weeks, at which time the antibody screen was negative. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank along with the cord blood sample from the baby and a request for postpartum RhIg.

ABO/Rh/Antibody Screen

ABO/Rh (gel method)				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	4+	0	4+	0
Antibody screen (AHG/gel method)				
SC1	1+			
SC2	1+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Test Results: Cord Blood

ABO/Rh (tube method)			
Baby's RBCs (forward typing)			
Anti-A	Anti-B	Anti-D	Weak D
0	2+	4+	NT
Cord Anti-IgG DAT: 0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel Panel

		Rh-hr							Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results					
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel	
1	R _{1w} R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	1+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	0	+	+	1+
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	0	+	0	0	0	0	+	0	+	+	+	0	+	1+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	+	0	+	1+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	0	+	0
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+	0	+	0
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	+	0
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	+	+	0	+	0
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	+	0	0	+	0	0	0	+	+	0
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	+	+	+	+	0	+	0
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	+	+	+	0	+	1+
Patient cell																													0	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Antibody titration and fetal screen	
Rh(D) antibody titer: 1	Fetal screen/rossette test: positive

Questions

1. What antibodies did you identify in the patient's sample?
2. What is the significance of the antibody titer result and the rosette test result?
3. What is the total dose of RhIg that the mother should receive if the Kleihauer–Betke (KB) test result is 3% (estimate total blood volume as 70 mL/kg)?
4. Suppose that the maternal anti-D titer turns out to be very high (e.g., titer of 256 or greater), and the baby requires exchange transfusion because of significant anemia and hyperbilirubinemia. If 400 mL of whole blood (based on 2.5 kg neonate weight and total blood volume of 80 mL/kg; note that neonatal exchanges are performed at twice the total blood volume) is required for the exchange, calculate the volume of citrate–phosphate–dextrose–adenine (CPDA) red blood cells (RBCs) and plasma that are needed in order to reconstitute an end product with 50% Hct. Use the following formula: $V_f = (C_i \times V_i)/C_f$, where V_f is the final volume of reconstituted whole blood product, C_i is the initial concentration (Hct) of the RBC unit (assume Hct of 70% for a CPDA unit), V_i is the initial volume of the RBC unit, and C_f is the final concentration (Hct) of the whole blood product (50%).
5. What special considerations are there for RBCs used for neonatal transfusions?
6. What considerations would there be if using an additive solution (ADSOL) RBC unit rather than a CPDA unit for the neonatal exchange transfusion?

Answers

1. **What antibodies did you identify in the patient's sample?** Anti-D is present.
2. **What is the significance of the antibody titer result and the rosette test result?** Since the titer of the anti-D is low (titer of 1), it is likely that the source of the antibody is from the prenatal RhIg. The positive rosette test, however, indicates that significant FMH may have occurred and that multiple RhIg doses may possibly be required based on the results of a quantitative test for FMH (i.e., KB or flow cytometry; see Chap. 21, question 5 answer).
3. **What is the total dose of RhIg that the mother should receive if the KB test result is 3% (estimate total blood volume as 70 mL/kg)?** To calculate this, first determine the mother's whole blood volume as $65 \text{ kg} \times 70 \text{ mL/kg} = 4550 \text{ mL}$. Then multiply $4550 \text{ mL} \times 0.03$ (3%) to get the volume of the fetal blood (136.5 mL). Divide 136.5 mL by 30 mL since each dose of 300 μg RhIg covers 30 mL of the fetal blood. This comes out to 4.55 doses. However, since the number to the right of the decimal is ≥ 5 , one would round up to five doses and add one dose as a margin of safety. Thus, the patient requires six doses of RhIg 300 μg in this case. To be complete, if the calculation came out to be 4.4 (rather than 4.5) doses, then one would round down and add one dose for total of five RhIg doses.
4. **Suppose that the maternal anti-D titer turns out to be very high (e.g., titer of 256 or greater), and the baby requires exchange transfusion because of significant anemia and hyperbilirubinemia. If 400 mL of whole blood (based**

on 2.5 kg neonate weight and total blood volume of 80 mL/kg; note that neonatal exchanges are performed at twice the total blood volume) is required for the exchange, calculate the volume of CPDA RBCs and plasma that are needed in order to reconstitute an end product with 50% Hct. Use the following formula: $V_f = (C_i \times V_i)/C_f$, where V_f is the final volume of reconstituted whole blood product, C_i is the initial concentration (Hct) of the RBC unit (assume Hct of 70% for a CPDA unit), V_i is the initial volume of the RBC unit, and C_f is the final concentration (Hct) of the whole blood product (50%). Using the above formula, we solve for V_i . $V_i = (V_f \times C_f)/C_i$. Thus, we come out with $V_i = (400 \text{ mL} \times 0.5)/0.7 = 285.7$ or 286 mL of CPDA RBCs. Since the total volume needed is 400 mL, subtracting 286 from 400, we get 114 mL of plasma. Therefore, we must mix 286 mL of the CPDA RBCs with 114 mL of plasma to make a reconstituted whole blood product of 400 mL with 50% Hct.

5. **What special considerations are there for RBCs used for neonatal transfusions?** In general, it is preferable to use RBCs that are fresh (i.e., not older than 7 days; note, however, that this is not supported by recent published AABB practice guidelines) [1], sickle hemoglobin (Hgb) negative, and cytomegalovirus (CMV) safe (either CMV seronegative and/or leukoreduced). In addition, it may be necessary to use irradiated RBCs (particularly in the case of a low-birth weight premature neonate or if the neonate had received intrauterine transfusion) to prevent transfusion-associated graft-versus-host disease (TA-GVHD). It is also preferable to use group O RBCs which are compatible with both the mother and the neonate. The RBCs must also be matched for any other maternal antibodies present; in this case, O-negative RBCs are necessary since maternal anti-D is the cause of the baby's anemia.
6. **What considerations would there be if using an additive solution (ADSOL) RBC unit rather than a CPDA unit for the neonatal exchange transfusion?** Additive solutions are commonly used to prepare RBC products nowadays because these solutions extend the shelf life over a CPDA unit from 35 to 42 days. However, the use of ADSOL presents unique issues for neonatal transfusions such as volume and concentration (ADSOL units have higher volumes and lower Hcts, typically 60%) as well as metabolic concerns (e.g., some ADSOL preparations contain mannitol which has increased risk of osmotic diuresis in the neonate).

Reference

1. Tobian AAR, Heddle NM, Wiegmann TL, Carso JL. Red blood cell transfusion: 2016 clinical practice guidelines from AABB. *Transfusion*. 2016;58(10):2627–30.

Recommended Reading

Josephson CD, Meyer E. Neonatal and pediatric transfusion practice. In: Fung MK, Grossman BJ, Hillyer CD, Westhoff CM, editors. Technical manual. 18th ed. Bethesda: AABB; 2014. p. 571–8.

Chapter 26

It May Do Harm



Clinical History

A 32-year-old woman presents to the obstetrical clinic at 8 weeks of pregnancy. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	W+			
SC2	W+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction), W weak

Gel Panel

		Rh-hr						Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results						
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel	
1	R _W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	0	0	+	0	
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	⁺ S	0	+	I+	
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	+	0	+	W+	
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	0	+	0	+	0	
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	⁺ W	0	+	0	0	
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	0	0	+	0	+	⁺ S	0	+	I+	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	0	⁺ S	0	+	W+	
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	+	+	0	+	0	0	+	0	0	
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	⁺ W	0	+	W+	W+	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	+	+	0	+	I+	0
Patient cell																														

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Tube and Dithiothreitol (DTT) Panel

		Rh-hr								Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: LAT/tube							
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Jk ^a	Jk ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	RT	AHG	CC	DTT	
Cell #	Rh-hr																																
1	R ₀ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	+	0	+	+	+	+	0	+	0	+	+	+	+	+	0	+	0	2+	0	0
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	+	+	0	+	0	+	+	+	+	0	0	+	W+	NT	W+	
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	+	0	+	+	+	+	0	0	0	+	0	+	+	+	+	0	1+	NT	1+	
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	+	0	0	0	+	+	+	+	+	0	W+	NT	W+		
5	r ⁺ r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	0	0	+	+	+	+	0	0	2+	NT	NT	
6	r ⁺ r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	+	0	W+	NT	W+	
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	+	+	+	+	0	+	0	+	+	+	+	+	0	1+	NT	1+		
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	+	+	+	+	0	W+	NT	W+		
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	0	0	0	0	2+	NT	NT	
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	+	0	+	+	+	+	+	+	0	W+	NT	W+	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	+	0	+	+	+	+	0	+	0	+	+	+	+	+	0	1+	NT	1+		NT
Patient cell																				0								0	0	2+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What antibodies did you identify?
2. Can you explain the difference in the reaction strength of the antibodies (i.e., W+ vs. 1+) seen on the panels?
3. What information does testing with dithiothreitol (DTT) provide in this case?
4. What recommendations regarding further antibody testing would you make for this patient in consideration of her pregnancy?

Answers

1. **What antibodies did you identify?** Anti-M antibody is present.
2. **Can you explain the difference in the reaction strength of the antibodies (i.e., W+ vs. 1+) seen on the panels?** The antibody is expressing a dosage effect, that is, the reactions are stronger with homozygous M antigen (i.e., M-positive/N-negative) cells and weakly or not at all with the heterozygous M antigen (i.e., M-positive/N-positive) cells. See Chap. 5, question 3 answer, for more information about dosage effect.
3. **What information does testing with DTT provide in this case?** DTT is a reducing agent that cleaves disulfide bonds, including those that hold immunoglobulin (Ig)M pentamers together. Thus, DTT differentiates IgM from IgG reactivity. While most antibodies to M antigen are cold reacting (IgM), in this case, because reactivity is maintained after treatment of the patient's sample with DTT, the reactions are due to IgG antibody rather than IgM. In addition to the fact that room temperature testing is negative, this is indicative of a warm-reacting, clinically significant anti-M, capable of causing hemolytic disease of the fetus/newborn (HDFN). Cases of HDFN caused by anti-M have been reported in the literature [1].
4. **What recommendations regarding further antibody testing would you make for this patient in consideration of her pregnancy?** In light of the above discussion, the patient has a warm-reacting IgG alloanti-M (note that the patient types as M-negative on the panel). Thus, the patient should be carefully monitored during pregnancy with anti-M titers, and fetal monitoring may become necessary.

References

1. Bajpayee A, Dubey A, Sonker A, Chaudhary RK. A case of severe foetal anaemia due to anti-M isoimmunisation salvaged by intrauterine transfusions. *Blood Transfus.* 2014;12(Suppl 1):s302–4.

Recommended Reading

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Chapter 27

Fuggedaboutit



Clinical History

A 72-year-old woman with a history of multiple pregnancies but no transfusions and no history of alloantibodies is admitted for workup of multiple myeloma and anemia, Hgb level 6.5 g/dL. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank along with a request for two units of irradiated red blood cells (RBCs).

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

Four days after the transfusion of the two units of irradiated RBCs, the patient's total bilirubin is noted to be elevated, while the Hgb level has dropped from 8.2 to 6.7 g/dL. The lactate dehydrogenase (LDH) level is also elevated (1100 U/L). A posttransfusion sample (EDTA anticoagulant) is submitted for transfusion reaction workup.

Test Results: Posttransfusion Sample

Clerical check			
Patient: O-positive		Donor units	
		Unit #1	O-positive
		Unit #2	O-negative
Visual check: No hemolysis			
	Polyspecific	Anti-IgG	Anti-C ₃ d
DAT (pre-sample)	0	NT	NT
DAT (post-sample)	2+	2+	0

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Acid Eluate Panel

Cell #	Rh-hr	Rh-hr							Kell				Duffy		Kidd		Lewis		MNS		P	Lutheran		Test results: IAT/tube PEG						
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	NT
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	+	+	0	0	+	+	+	0	0	+	0	0	NT
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	+	+	^s	0	+	0	NT
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	+	0	+	3+	NT
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	0	+	+	+	0	+	3+	NT
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	^w	0	+	3+	NT	
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	0	0	+	+	^s	0	+	3+	NT	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	^s	0	+	3+	NT		
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	+	0	0	0	+	0	0	0	+	3+	NT	
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	^w	0	+	3+	NT		
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	+	+	0	+	0	0	NT
Last wash SC1																													0	2+
Last wash SC2																													0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Gel Panel

		Rh-hr						Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results						
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N		S	s	P ₁	Lu ^a	Lu ^b	
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	+	0	0	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	+	0	0	
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	+	+	0	0	
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	0	2+	2+	
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	+	2+	2+	
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	+	+	+	2+	2+	
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	0	0	+	0	+	+	+	2+	2+	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	+	+	+	2+	2+	
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	+	0	+	0	+	0	+	2+	2+	
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	+	+	2+	2+	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	+	+	+	0	0	0
Patient cell			2+	0	0	2+																						2+		

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Selected-Cell (“Rule-Out”) Panel

		Rh-hr								Kell						Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results:				
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	37 °C	AHG	CC	
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	0	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	+	+	0	0	+	0	0	2+	
3	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	+	0	0	0	0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What antibodies did you identify in the eluate and the panel?
2. Why is the patient's antibody screen negative?
3. Considering that the patient experienced a delayed hemolytic reaction, why were both transfused RBC units compatible by crossmatch?
4. What is the most likely Rh genotype (Wiener notation) in this Caucasian patient based on the patient's phenotype?
5. Assuming that the patient had all four of her children with the same Caucasian, Rh-positive father and that she developed the antibody through pregnancy exposure, what is the most likely Rh genotype (Wiener notation) of the father?
6. Given that the patient received an O-positive and an O-negative RBC unit and that only one of the two units was found to be incompatible with the patient's pretransfusion sample by antihuman globulin (AHG) crossmatch on further workup, which of the two units most likely is responsible for the delayed hemolytic reaction? Why?
7. Why were irradiated RBCs indicated for this patient?

Answers

1. **What antibodies did you identify in the eluate and the panel?** Anti-f antibody is present. However, in a manner not unlike differentiation between anti-E-like antibody and anti-hr^B (see Discussion in Chap. 9, question 1 answer), anti-hr^S antibody may on rare occasions be mistaken for anti-f antibody; anti-hr^S, though, like anti-hr^B, is generally not considered to be clinically significant [1]. Anti-f antibody, on the other hand, may cause delayed hemolytic transfusion reactions and hemolytic disease of the fetus/newborn (HDFN) [2].
2. **Why is the patient's antibody screen negative?** Although the f antigen is a prevalent compound antigen (composed of ce antigens), it is absent on both of the screening cells (refer to screening cell antigen profile in the beginning of the book); the f antigen is found in 65% of Caucasians and 92% of African Americans [3].
3. **Considering that the patient experienced a delayed hemolytic reaction, why were both transfused RBC units compatible by crossmatch?** Since the patient did not have a history of clinically significant alloantibodies and the current antibody screen is negative, the RBC units would have been crossmatched by the immediate-spin (IS) or the electronic (computer) crossmatch (assuming that at least two ABO typings were done) methods, both of which are only capable of detecting ABO incompatibility (see Chap. 3 for discussion about crossmatch methods).
4. **What is the most likely Rh genotype (Wiener notation) in this Caucasian patient based on the patient's phenotype?** The patient's genotype most likely

is R_1R_1 , given that the patient is Rh(D)-antigen positive and is positive for the Rh(C) and R(e) antigens. Note that r' (Ce) haplotype is rather uncommon [3]. See Chap. 2, question 3 answer, for information about Rh haplotypes.

5. **Assuming that the patient had all four of her children with the same Caucasian, Rh-positive father, what is the most likely Rh genotype (Wiener notation) of the father?** Given that at least one child would have had to inherit the f antigen (ce) from the father in order for the patient to become exposed and sensitized to the antigen during pregnancy, the father could either be R_1r , R_2r or R_0r . Of these, R_1r is most common in the Caucasian population (31% vs. 10% and 3%, respectively) [3].
6. **Given that the patient received an O-positive and an O-negative RBC unit and that only one of the two units was found to be incompatible with the patient's pretransfusion sample by AHG crossmatch on further workup, which of the two units is most likely responsible for the delayed hemolytic reaction? Why?** It is highly probable that the Rh-negative unit caused the reaction given that anti-f is essentially anti-ce as explained above; for Rh-negative, r haplotype (ce) is much more prevalent than either r' (Ce), r'' (cE), or r^y (CE). On the other hand, for Rh-positive, R_1R_1 and R_1R_2 genotypes are fairly common (17% and 11% in Caucasians, respectively) and would not produce RBCs with the ce phenotype [3].
7. **Why were irradiated RBCs indicated for this patient?** Given that the patient is being worked up for a hematologic malignancy (multiple myeloma) and may be sufficiently immunosuppressed, the patient would be considered at risk for transfusion-associated graft-versus-host disease (TA-GVHD) and, therefore, should be transfused with irradiated cellular products. Gamma irradiation prevents donor T-lymphocytes from mounting an immune attack on host tissues in susceptible patients.

References

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Chapter 28

Decisions, Decisions!



Clinical History

A 38-year-old woman presents to the emergency department (ED) with acute vaginal bleeding and symptomatic anemia, hemoglobin (Hgb) 6.0 g/dL. The patient has a history of uterine fibroids and chronic anemia. She received a transfusion of red blood cells (RBCs) at your hospital 1 year ago after uterine myomectomy surgery; the antibody screen was positive at that time, and the patient was found to have anti-E and anti-Fy^a alloantibodies. An ethylenediaminetetraacetic acid (EDTA) anti-coagulant sample is submitted to the blood bank for type and screen along with an order for crossmatch of two RBC units for transfusion.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	0	0	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	2+			
SC2	3+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction); W weak

Gel Panel

		Rh-hr							Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results					
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	S	P ₁	Lu ^a	Lu ^b	AHG gel	
1	R _{1w} R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	1+	
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	+	+	0	+	3+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	+	0	+	0
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	+	0	+	0
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+	0	+	3+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	0	0	+	0	+	+	+	0	+	W+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	0	+	+	0	+	1+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	0
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	+	0	+	+	+	+	+	0	+	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	+	+	+	0	+	W+
Patient cell																														0

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Tube Panel

Cell #	Rh-hr	Rh-hr							Kell				Duffy	Kidd	Lewis	MNS			P	Lutheran	Test results											
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a				Kp ^b	Js ^a	Js ^b			Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a
1	R ₁ 0R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	+	0	0	0	0	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	+	0	+	0	+	0	+	+	+	0	1+	0	1+	0	NT
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	+	+	2+	2+	3+	NT	NT
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	+	0	1+	0	0	0	NT
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	+	+	0	0	0	2+	2+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	0	1+	2+	3+	NT	NT
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	0	+	+	0	+	+	+	+	+	0	0	0	0	2+	2+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	+	+	+	+	+	0	1+	0	1+	NT	NT
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	0	+	0	0	+	0	+	+	+	0	0	0	0	2+	2+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	+	+	0	0	0	2+	NT	NT
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	0	2+	0	0	2+	2+
Patient cell																							0					0	0	0	2+	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Additional History

As the blood bank technologist is working to screen for antigen-compatible RBC units for crossmatch, a call is received from the ED physician requesting stat RBC transfusion (uncrossmatched RBCs); the physician states that the patient has become hypotensive and unresponsive to fluid bolus and is experiencing chest pain with ischemic electrocardiogram (ECG) changes (i.e., demand ischemia). Meanwhile, the blood bank inventory of Rh-negative RBC units is very low due to a severe blood shortage in the region: in fact, the blood bank is down to its last seven units of O-negative and five units of A-negative RBCs (whereas the typical inventory for the blood bank is 20–25 units of O-negative and 15–20 units of A-negative RBCs).

Questions

1. What antibodies did you identify?
2. Given the situation regarding the severe anemia and active bleeding in the patient who has become unstable, how would you handle the stat request for uncrossmatched RBCs?
3. After the initial stat transfusion of uncrossmatched RBCs, how should a subsequent request for RBC transfusion of one unit be handled given the following additional information about the case? The patient has been admitted to the hospital, and a hospitalist physician states that the patient's ECG ischemic changes have resolved and that she is no longer experiencing chest pain 1 day after the RBC transfusion; myocardial infarction was ruled out by normal serial troponin I levels. However, the patient remains tachycardic with heart rate of 122 beats/min, and she is quite pale and weak. The posttransfusion Hgb is 6.8 g/dL. The hospitalist further relates that the patient has two healthy children, does not desire future pregnancy, and, in fact, has discussed hysterectomy surgery with her gynecologist as a result of the chronic uterine bleeding she has experienced. Meanwhile, further testing in the blood bank has found that the transfused RBC unit is crossmatch-compatible (AHG crossmatch) and, fortunately, that the unit is negative for both the Rh(E) and the Fy^a antigens. In light of this, would you recommend transfusion of Rh(D)-positive RBCs?

Answers

1. **What antibodies did you identify?** The anti-E and anti-Fy^a alloantibodies, previously identified in your blood bank, are evident in the patient's current sample. A new anti-M antibody, reactive at room temperature (RT), is also present.
2. **Given the situation regarding the severe anemia and active bleeding in the patient who has become unstable, how would you handle the stat request for**

uncrossmatched RBCs? Since the patient has become medically unstable with ongoing bleeding, stat RBC transfusion of uncrossmatched blood is necessary. It is unreasonable to delay transfusion in order to find fully antigen-compatible RBC units in accordance with the patient's identified alloantibodies. Of the three alloantibodies, though, only the anti-E and anti-Fy^a antibodies are clinically significant; anti-M is shown to be a cold-temperature reactive, immunoglobulin (Ig) M and not likely to cause in vivo hemolysis. Since the patient in this case is Rh(D)-negative (group A-negative), a fairly straightforward way of ensuring Rh(E)-antigen-negative RBCs would be to select Rh(D)-negative (either A-negative or O-negative) RBCs for transfusion since the vast majority of Rh(D)-negative RBCs are also Rh(E)-negative (recall that the r'' [cE] and r' [CE] haplotypes are uncommon with a combined prevalence of about 1%; see Chap. 2, question 3 answer for review of this topic) [1]. However, selection of Rh(D)-negative RBCs would not account for the Fy^a antigen which would be expected to have a frequency greater than 60% in the donor population (with a frequency of over 65% in Caucasians versus less than 15% in African Americans) [2]. Thus, there is a fairly high chance that the patient will be exposed to Fy^a antigen, even with transfusion of a single unit, and may develop a delayed hemolytic transfusion reaction. Therefore, the patient should be closely monitored after the transfusion until the Fy^a-antigen status of the unit (along with E antigen) can be determined and an antihuman globulin (AHG) crossmatch performed to verify compatibility.

3. **After the initial stat transfusion of uncrossmatched RBCs, how should a subsequent request for RBC transfusion of one unit be handled given the following additional information about the case?** The patient has been admitted to the hospital, and a hospitalist physician states that the patient's ECG ischemic changes have resolved and that she is no longer experiencing chest pain 1 day after the RBC transfusion; myocardial infarction was ruled out by normal serial troponin I levels. However, the patient remains tachycardic with heart rate of 122 beats/min., and she is quite pale and weak. The posttransfusion Hgb is 6.8 g/dL. The hospitalist further relates that the patient has two healthy children, does not desire future pregnancy, and, in fact, has discussed hysterectomy surgery with her gynecologist as a result of the chronic uterine bleeding she has experienced. Meanwhile, further testing in the blood bank has found that the transfused RBC unit is crossmatch-compatible (AHG crossmatch) and, fortunately, that the unit is negative for both the Rh(E) and the Fy^a antigens. **In light of this, would you recommend transfusion of Rh(D)-positive RBCs?** Given that the acute crisis (i.e., cardiac ischemia) has resolved after the transfusion of one RBC unit, there is now the luxury of time to screen for additional antigen-compatible RBC units (i.e., E- and Fy^a-antigen negative) for transfusion to the patient; transfusion of RBCs may be of benefit since there are ongoing signs and symptoms of anemia. The practical decision then is whether to crossmatch Rh(D)-positive RBCs for this Rh-negative patient in light of the severe blood shortage and substantially low Rh-negative inventory that the blood bank is experiencing. Although the patient is a relatively young female still within the age of childbearing years, she has

expressly stated that she does not wish to get pregnant again and is planning for hysterectomy surgery. Therefore, transfusion of Rh-positive RBCs (R_0 or R_1 cells, which are E-negative) may be feasible as a means to conserve Rh-negative inventory. However, since this patient is a known antibody producer (i.e., the patient already has made alloantibodies to the E and Fy^a antigens), there is a high risk that the patient will make anti-D after exposure to the Rh-positive cells, so discussion with the patient regarding this risk and future pregnancy is essential. On the other hand, since the patient may only need one additional unit of RBCs and no further transfusion during the hospital admission, it may be best practice to transfuse an Rh-negative unit to prevent Rh(D) sensitization which could complicate transfusion therapy in the future should another crisis arise as well as any future pregnancy, which nevertheless remains a possibility until the hysterectomy surgery is performed.

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Chapter 29

Oh! Are You My Type?



Clinical History

A 22-year-old man is brought into the emergency department (ED) after sustaining trauma resulting in a fracture of the right femur sustained during a skiing accident. An ethylenediaminetetraacetic acid (EDTA) anticoagulant sample is submitted to the blood bank for type and screen and crossmatch of four red blood cell (RBC) units. No transfusion history is given.

ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	3+	4+	4+
<i>Antibody screen (tube LISS method)</i>				
		37 °C	AHG	CC
SC1		4+	4+	NT
SC2		4+	4+	NT

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Tube Panel

Cell #	Rh-hr	Rh-hr								Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: LAT/Tube LISS							
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	IS	37 °C	AHG	CC	
1	R ₁ wR ₁	+	+	0	0	+	0	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	+	0	+	4+	4+	NT	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	+	+	+	0	0	0	+	+	+	+	0	0	0	+	4+	4+	NT	
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	0	+	0	+	+	+	+	0	0	0	0	0	+	0	+	+	+	0	+	4+	4+	NT	
4	R ₀ r	+	0	0	+	+	+	0	+	0	0	+	0	+	0	0	0	0	0	0	0	0	+	+	+	+	+	0	+	4+	4+	NT	
5	r'r	0	+	0	+	+	+	0	0	0	0	+	0	+	0	+	0	+	0	0	+	0	0	+	0	+	+	0	+	4+	4+	NT	
6	r''r	0	0	+	+	+	+	0	0	0	0	+	0	+	+	0	+	+	+	0	0	0	+	+	+	+	+	0	+	4+	+	4+	NT
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	+	0	+	4+	4+	NT	
8	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	+	+	+	0	+	+	0	0	+	+	+	+	+	0	+	4+	4+	NT	
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	0	+	0	+	0	0	0	+	4+	4+	NT	
10	rr	0	0	0	+	+	+	0	0	0	0	+	0	+	+	0	+	0	+	0	0	+	+	+	+	+	+	0	+	4+	4+	NT	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	0	+	0	+	0	+	0	+	0	0	0	+	+	0	+	0	+	0	+	4+	4+	NT	
Patient cell																													0	0	0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Additional History

The blood bank technologist on duty suspects a cold agglutinin and performs the preceding panel. She further crossmatches four units of group O-positive RBCs by full crossmatch using pre-warm technique, results below.

Crossmatch Results

AHG crossmatch results		
	AHG	CC
Donor unit #1	4+	NT
Donor unit #2	4+	NT
Donor unit #3	4+	NT
Donor unit #4	4+	NT

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Further History

During the preceding antibody workup, a stat request for two units of uncross-matched group O-negative RBCs is received from the ED, prompting the transfusion service medical director to contact the ED physician. The ED physician indicates that the patient is tachycardic but not hypotensive with a hematocrit (Hct) of 30%. The physician is concerned about further bleeding and the need to take the patient to surgery. He also notes that the patient has no underlying medical conditions, is not taking any medications or herbal supplements, and has never been transfused based on history obtained from the patient’s family via telephone. Finally, the ED physician believes that the patient is of Indian (Asian) descent based on the patient’s surname. Based on this additional information, the transfusion service medical director recommends withholding immediate transfusion and orders blood samples to be urgently sent off to the immunohematology reference laboratory at the nearby blood center for investigation with stat result received as below:

ABO/Rh (tube method)				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	3+	4+	4+
Anti-H lectin	Patient: 0	SC1: 4+		
Antibody screen (tube LISS method)				
	37 °C	AHG	CC	
SC1	4+	4+	NT	
SC2	4+	4+	NT	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Questions

1. What is the patient's ABO/Rh blood type?
2. Why are all of the donor units incompatible, and what might have occurred had uncrossmatched O-negative blood been transfused to the patient?
3. How would you manage this patient's anemia?
4. What would you recommend to the patient for future precaution once the patient recovers from this event?

Answers

1. **What is the patient's ABO/Rh blood type?** Based on the initial ABO/Rh results, the patient appears to be group O, Rh-positive. Refer to Chap. 1, question 1 answer for further information on forward and reverse ABO typing. However, given that all group O cells were strongly incompatible by crossmatch, the transfusion service medical director in this case suspected that the patient's blood type may actually be the rare Bombay phenotype (also known as O_h and discovered in Bombay, India, in 1952). This was confirmed by the patient's negative anti-H lectin (*Ulex europaeus*) result. O_h is rare but occurs in 1 in 10,000 individuals from India, though it may also be found in individuals with European ethnicity (extremely rare) [1]. H antigen (fucose) is a separate blood group system apart from ABO that is inherited by genes located on chromosome 19 (ABO genes are located on chromosome 9); actually, the genes encode for two fucosyltransferases known as *FUT1* (H gene, type 2 chain H on RBCs) and *FUT2* (secretor [*Se*] gene, type I chain H and Le^b antigen in secretions; soluble H antigen) [2]. Para-Bombay types, homozygous for a nonfunctional H gene (*hh*) but having at least one functional *Se* gene, also exist and can express weak A and/or B antigens on RBCs adsorbed from plasma type I chains [2].
2. **Why are all of the donor units incompatible, and what might have occurred had uncrossmatched O-negative blood been transfused to the patient?** The patient likely would have suffered from an acute (intravascular) hemolytic transfusion reaction given that Bombay phenotypes contain a potent anti-H that is hemolytic at a wide range of temperatures and can activate complement. As a result of this hemolytic anti-H, group O cells, which are all rich in H antigen, are incompatible.
3. **How would you manage this patient's anemia?** Unfortunately, this patient, like all Bombay phenotypes, must receive O_h blood which is obviously not readily available; it must be acquired from rare donor registries and would be dispensed as frozen-thawed RBCs. As such, the patient must be managed without the immediate availability of compatible blood. Consideration must be given to blood conservation strategies, including the use of intraoperative and postoperative cell salvage as well as acute normovolemic hemodilution techniques should

the patient require surgical repair of the fracture, in addition to use of oxygen-carrying therapeutic agents (i.e., artificial blood manufactured by purifying bovine hemoglobin and currently available via compassionate use only as they are not approved by the US Food and Drug Administration [FDA]) [3]. Furthermore, the patient's family, especially siblings, should be checked for O_h blood type and asked to urgently donate blood as able.

4. **What would you recommend to the patient for future precaution once the patient recovers from this event?** Once the patient recovers fully and is in good health, he should be encouraged to donate his blood for frozen storage (many donor centers will accommodate this) in case of future need. His donated blood may also be used for the rare donor program (should the patient agree to this) so that other Bombay phenotypes may benefit as well should the need arise for blood transfusion. RBCs stored frozen (at -80°C or below), by use of glycerol as a cryoprotectant agent, may be thawed and deglycerolized for transfusion up to 10 years later. However, they generally must be transfused within 24 h after thawing unless a closed system has been used for the thaw process [4]. Family members, if type O_h and in good health, may also be encouraged to continue annual blood donations.

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Clinical History

A 16-year-old African American male with sickle cell anemia is admitted to the hospital with worsening chest pain, shortness of breath, and fever (temperature 39.0 °C). Bilateral lower lobe infiltrates are seen on chest X-ray, and the patient’s oxygen saturation on room air is 88%. The patient’s hemoglobin (Hgb) level is 6.2 g/dL (his baseline Hgb is 7.5 g/dL). The patient has not been seen in your hospital prior to this admission, but the patient does report a history of blood transfusions, including several units of red blood cells (RBCs) transfused 2 months ago for severe pain while traveling abroad. An ethylenediaminetetraacetic acid (EDTA) anticoagulant sample is submitted to the blood bank for type and screen.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	4+	0	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	3+			
SC2	3+			

Reaction scale = 0 (no reaction) to 4 + (strong reaction); W weak

Gel Panel

		Rh-rh							Kell							Duffy		Kidd		Lewis		MNS				P	Lutheran	Test results		
Cell #	Rh-hr	D	C	E	c	e	f	c ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel	
1	R _{1w} R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	3+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	0	+	+	3+
3	R ₂ R ₂	+	+	0	+	0	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	0	0	+	0	+	s ₊	0	+	3+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	+	0	0	+	+	+	+	+	0	+	3+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	0	+	+	+	0	+	3+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	+	+	w ₊	0	+	+	3+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	s ₊	0	+	+	3+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	+	s ₊	0	+	+	3+
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	0	+	0	+	+	+	0	0	+	0	0	0	0	0	+	+	3+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	+	w ₊	0	+	+	3+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	0	+	+	0	+	3+
Patient cell																														3+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

DAT profile			
Polyspecific:	3+	Anti-IgG:	3+
		Anti-C ₃ d	0

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Acid Eluate Panel

		Rh-hr										Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/Tube			
Cell #	Rh-hr	D	C	E	c	e	f	c ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC	
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	3+	NT
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	3+	NT	
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	s ₊	0	+	3+	NT	
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	0	+	3+	NT	
5	r ^r r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	+	0	+	3+	NT
6	r ^r r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	w ₊	0	+	3+	NT	
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	s ₊	0	+	3+	NT	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	0	s ₊	0	+	3+	NT	
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	+	0	0	0	0	0	0	0	+	3+	NT	
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	0	+	+	+	w ₊	0	+	3+	NT	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	+	0	+	3+	NT	
Last wash SC1																													0	2+	
Last wash SC2																													0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Additional History

The patient is treated with pain medications, antibiotics (for possible pneumonia), intravenous (IV) fluids, and supplemental oxygen by nasal cannula. An RBC exchange transfusion is also considered the following day in light of the patient's hypoxemia and the possibility of acute chest syndrome as well as the desire to avoid intubation. Meanwhile, in further workup of the patient's antibody panel results, the blood bank has sent an EDTA anticoagulant sample to the regional immunohematology reference laboratory and receives a report confirming the presence of a warm-reactive autoantibody; the presence of an alloanti-Jk^a antibody is also reported, while alloanti-S antibody cannot be excluded (based on differential alloadsorption technique). The immunohematology report also notes that phenotyping of the patient's RBCs (by serologic testing) was not performed due to the recent RBC transfusions.

Questions

1. Based on the clinical scenario, how would you manage this patient's RBC transfusion needs? Is RBC exchange transfusion indicated?
2. In consideration of the immunohematology reference laboratory report, what is the best method to identify RBC antigens present on the patient's red cells?
3. What is the molecular basis for the ABO and Rh blood group antigens?
4. What molecular techniques are available for RBC antigen testing?
5. What are the major applications of deoxyribonucleic acid (DNA)-based assays in immunohematology?

Answers

1. **Based on the clinical scenario, how would you manage this patient's RBC transfusion needs? Is RBC exchange transfusion indicated?** Given that this sickle cell patient has a warm autoantibody, least-incompatible RBCs (see Chap. 9, question 7 answer for a discussion on this topic) that are also compatible for the identified (anti-Jk^a) and suspected (anti-S) alloantibodies (based on differential alloadsorption, see Chap. 17 for more information on this topic) must be crossmatched for transfusion. The crossmatched RBCs must also be sickle Hgb negative, and it is common practice by many transfusion services to provide extended antigen matched RBCs (typically matched for Rh and K antigens; see Chap. 18, question 3 answer for discussion on this topic). However, provision of extended antigen matched RBCs is complicated in this case since serologic phenotyping was not performed as a result of the recent RBC transfusions (though

one could reasonably presume that the patient is K-antigen negative based upon the fact that the antigen is present in only 2% of African Americans [see Table of RBC Antigen Frequencies in front section of this workbook]). Meanwhile, RBC exchange transfusion may be indicated for sickle cell patients who are experiencing a severe crisis or, in some cases, prophylactically to prevent recurrence of a catastrophic acute event (such as a stroke). Thus, RBC exchange transfusion, which has the advantage over simple transfusion of increasing the Hgb A to Hgb S ratio while maintaining the hematocrit level at or below 30% and minimizing the increase of iron stores, may be performed in this patient for treatment of acute chest syndrome. The American Society for Apheresis (ASFA) classifies RBC exchange transfusion as a category II indication (disorders for which apheresis is accepted as second-line therapy, either as a standalone treatment or in conjunction with other modes of treatment) for treatment of acute chest syndrome in sickle cell patients [1].

2. **In consideration of the immunohematology reference laboratory report, what is the best method to identify RBC antigens present on the patient's red cells?** RBC antigen phenotyping is notably unreliable in a patient who has been recently (i.e., within the prior 3 months) transfused. Although there has been a technique described for rapidly isolating patient sickle cells via osmotic lysis of transfused donor cells using hypotonic saline [2], the most reliable method for determining the RBC antigen profile under such circumstances is via genotyping. The presence of donor RBCs does not interfere with molecular assays used for genotyping since DNA is extracted from white blood cells (WBCs). Since the expression of many clinically significant RBC antigens on human RBCs is determined by single nucleotide polymorphisms (SNPs), detection of these SNPs can predict the RBC phenotype as an alternative to serological typing [3]. Although genotyping is cost-effective and efficient, unfortunately, a major limitation of the use of genotyping is the turnaround time for results, which can be several days or more, especially since most hospital blood banks do not have the capacity to perform the testing on-site. In addition, although DNA-based testing can provide a fairly accurate predictive phenotype, it is possible that the genotype determination will not correlate with RBC antigen expression owing to genetic events that may silence or weaken expression of antigens encoded by an allele or false-negative results when the antigen is expressed due to alterations in the probe/primer binding site [4].
3. **What is the molecular basis for the ABO and Rh blood group antigens?** The patient's blood type is group A, Rh-positive (see Chap. 1, question 1 answer for more information on this topic). In the ABO blood group system, the *A* allele encodes for the $\alpha(1,3)$ -*N*-acetylgalactosaminyltransferase, and the *B* allele encodes for the $\alpha(1,3)$ -galactosyltransferase. In an individual with the AB phenotype, the A and B transferases coexist. The O phenotype is formed by a mutation which causes a nonfunctional transferase enzyme. The Rh antigens are encoded by the *RHD* and *RHCE* genes which each contain 10 exons, and they have a 97% nucleotide sequence homology. The *RHD* allele encodes for the Rh(D) antigen, while the *RHCE* allele encodes for the Rh(C), Rh(c), Rh(E), and

Rh(e) antigens. These antigens have similar polypeptides of 417 amino acids with 12 membrane-spanning domains. These proteins form a complex with Rh-associated glycoprotein (RhAG) on the RBC membrane. Although there are over 60 antigens identified within the Rh blood group system, the most common and tested in clinical practice are D, C, c, E, and e.

4. **What molecular techniques are available for RBC antigen testing?** There are multiple molecular techniques which can be used to genotype blood group antigens depending on the laboratory capabilities and experience. Many of these techniques are based on polymerase chain reaction (PCR) which is an amplification method for generating nucleic acid fragments for direct analysis. PCR setup requires several specialized components: a DNA template which contains target DNA, DNA polymerase enzyme that amplifies the target region, two DNA primers that are complementary to the sense and antisense strands of the DNA target, deoxynucleoside triphosphates (dNTPs, the molecules which the DNA polymerase synthesizes a new DNA strand from), and a thermal cycler. Modified PCR techniques, such as allele-specific PCR (AS-PCR) and multiplex PCR amplification, are commonly used in laboratories. In contrast to normal PCR primers, in AS-PCR, at least one of the primers is chosen from a polymorphic area. A matched primer will initiate reaction/replication but a mismatched primer will not. Depending on the primer, the appearance of an amplification product will indicate the genotype. Multiplex PCR amplification is another DNA-based assay which can be used for genotyping of blood groups. This is a faster and more efficient amplification method of blood group antigens. This PCR method amplifies several different target DNA sequences simultaneously by using multiple primers. Then, to predict RBC phenotype, the PCR product can be used for hybridization to RBC antigen allele-specific oligonucleotide probes. Finally, next-generation sequencing (NGS, also known as massively parallel sequencing [MPS]) technology has allowed for faster throughput and greater accuracy of gene sequencing and has been found to be useful for RBC antigen genotyping [5, 6].
5. **What are the major applications of DNA-based assays in immunohematology?** There are many potential uses for molecular DNA-based testing in the blood bank laboratory. These include testing to obtain a predictive phenotype of:
 - (a) Fetal RBCs
 - (b) RBCs in a recently transfused patient
 - (c) Antigens for which typing antisera may not be available (e.g., anti-Do^a, anti-Do^b, anti-Js^a, anti-V, anti-VS)
 - (d) Patient RBCs to distinguish an alloantibody from an autoantibody
 - (e) Patient RBCs after allogeneic stem cell transplantation
 - (f) RBCs coated with antibodies (i.e., a positive immunoglobulin [Ig]G direct antiglobulin test [DAT])
 - (g) RBCs when the serologic results are unusual or discrepant

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Chapter 31

Bad Medicine



Clinical History

A 78-year-old male patient is transferred from the nursing home to the hospital for treatment of pneumonia. The patient has a past medical history that is significant for hypertension, congestive heart failure, type 2 diabetes mellitus, and gastroesophageal reflux disease (GERD) for which the patient takes amlodipine besylate, hydrochlorothiazide, metformin, and omeprazole, respectively. The patient has been treated in the past for pneumonia with intravenous (IV) antibiotics. The current sputum culture is positive for *Streptococcus pneumoniae*, and the patient is started on IV ceftriaxone. In addition, acetaminophen is given to control the patient's fever (temperature 101.2°F on admission). On the fifth hospital day, the patient is noted to have worsening anemia (drop of hemoglobin [Hgb] level from 10.5 to 7.2 g/dL) with elevation of total bilirubin (6.2 mg/L), lactate dehydrogenase (LDH, 2100 U/L), and reticulocytes (10.3%). In addition, the haptoglobin is noted to be less than 7 mg/dL. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank along with a request for two units of RBCs and a direct antiglobulin test (DAT) profile for workup of hemolytic anemia.

ABO/Rh/Antibody Screen

ABO/Rh (gel method)				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
3+	0	4+	1+	4+
Anti-A ₁ lectin	Patient: 0	A ₁ cells: 4+	A ₂ cells: 0	
Antibody screen (AHG/gel method)				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

DAT Profile and Acid Eluate Panel

DAT profile				
Polyspecific:	3+	Anti-IgG:	2+	Anti-C ₃ d: 3+

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Acid Eluate Panel

		Rh-hr										Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/Tube PEG		
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	0	2+
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	0	+	0	0	0	0	+	0	+	+	0	+	0	2+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	+	0	+	0	2+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	+	0	0	+	+	+	0	+	0	2+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	+	0	0	+	+	+	+	0	+	0	2+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	0	+	0	2+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	0	+	+	0	+	0	2+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	0	+	0	0	+	0	+	0	+	0	0	+	0	2+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	+	+	0	+	0	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	0	+	0	0	0	+	+	0	+	+	0	+	0	2+
Last wash SC1																													0	2+
Last wash SC2																													0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What is the patient's ABO/Rh type?
2. In light of the patient's ABO front and back type discrepancy, what ABO type would you crossmatch for this patient?
3. What are the possible causes of the patient's positive DAT in light of the negative eluate panel?
4. In light of the patient's medical history and medications, is drug-induced hemolytic anemia (DIHA) a consideration? If so, which of the medications is the most likely to be causative? What testing would be necessary to prove medication as the cause of the hemolytic anemia?

Answers

1. **What is the patient's ABO/Rh type?** The patient is an A₂ type with an anti-A₁ in the back type. Note that the anti-A₁ lectin (*Dolichos biflorus*) is negative in this case.
2. **In light of the patient's ABO front and back type discrepancy, what ABO type would you crossmatch for this patient?** Given that anti-A₁ antibodies made by type A₂ persons are not usually clinically significant (i.e., they are cold reacting), crossmatching group A (i.e., group A₁) blood would be acceptable. However, since the anti-A₁ could interfere with the crossmatch, some might prefer to give group O blood instead.
3. **What are the possible causes of the patient's positive DAT in light of the negative eluate panel?** As noted in Chap. 13, question 1 answer, there are three possible causes of a positive immunoglobulin (Ig)G DAT with a negative eluate panel: (1) an antibody to A or B antigens (as in ABO hemolytic disease of the fetus/newborn, HDFN), since all panel cells are group O, (2) an antibody to a low-frequency antigen, and (3) a drug-induced antibody.
4. **In light of the patient's medical history and medications, is DIHA a consideration? If so, which of the medications is the most likely to be causative? What testing would be necessary to prove medication as the cause of the hemolytic anemia?** Given the patient's history of sepsis and treatment with IV antibiotics, and in light of the fact that the patient is currently receiving IV ceftriaxone and has a positive DAT with a negative eluate, it is possible that the patient is experiencing DIHA. In addition to the positive DAT, the elevated LDH and total bilirubin along with the low haptoglobin are also suggestive of hemolytic anemia, particularly intravascular hemolysis (note that DIHA mechanisms can involve extravascular and intravascular hemolysis depending on the involved medication and complement activation) [1]. Ceftriaxone is a third-generation cephalosporin that has been implicated in cases of DIHA, including fatal cases [2, 3]. DIHA is quite rare, but several mechanisms have been described to explain

how the drugs induce hemolysis. In general, these mechanisms fall into drug-dependent and drug-independent categories [1]. In the former, presence of the drug or drug metabolite is necessary in order for the antibody to be reactive in the eluate panel. In the latter, presence of the drug is not necessary for the eluate to be reactive; this has been described for the drug alpha-methyldopa. In this case, in order to prove ceftriaxone as the causative agent of the hemolytic anemia, it would be necessary to coat the panel cells with the medication in order to produce positive reactions with the patient's sample.

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Chapter 32

Passengers and Hitchhikers



Clinical History

A 54-year-old woman with hepatitis C cirrhosis, *status post* deceased-donor orthotopic liver transplant 14 days ago, returns for outpatient follow-up with complaints of excessive fatigue, lightheadedness, and dyspnea on exertion. The patient had experienced an uneventful postoperative course and was discharged from the hospital in good condition on postoperative day #4. Laboratory studies show that the hemoglobin (Hgb) has decreased from 9.1 (hospital discharge) to 5.1 g/dL. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) and a request for two units of red blood cells (RBCs) are submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A1 cells	B cells
0	0	3+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	2+			
SC2	2+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel Panel

Cell #		Rh-hr	Rh-hr										Kell						Duffy		Kidd		Lewis		MNS			P	Lutheran	Test results		
			D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel		
1		R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	0	+	2+	
2		R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	0	0	+	+	2+	
3		R ₂ R ₂	+	+	0	+	0	0	0	0	0	+	0	+	0	+	0	0	+	+	0	0	0	0	+	+	+	+	+	0	+	2+
4		R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	+	+	0	+	2+	
5		r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	+	0	+	3+	
6		r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	0	0	+	0	+	+	+	+	+	0	+	0	
7		rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	+	0	
8		rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	+	+	+	0	+	0	
9		rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	0	0	0	+	+	0	
10		rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	+	+	0	+	0	
11		R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+	2+	
Patient cell																															2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

DAT Profile and Acid Eluate Panel

DAT profile			
Polyspecific:	2+	Anti-IgG:	2+
		Anti-C ₃ d:	0

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Acid Eluate Panel

Cell #		Rh-hr										Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/Tube PEG			
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S		s	P ₁		Lu ^a	Lu ^b	AHG
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	2+	NT	CC
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	0	+	0	+	+	0	+	+	0	0	0	+	+	+	+	0	0	+	2+	NT	NT
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	+	0	+	2+	NT	NT
4	R ₀ r	+	0	0	+	+	0	+	0	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	0	+	2+	NT	NT
5	r ⁺ r	0	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	0	+	+	+	0	+	3+	NT	NT
6	r ⁺ r	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	+	+	0	+	0	2+	2+
7	rr	0	0	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	0	+	+	+	+	0	+	0	2+	2+
8	rr	0	0	0	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	0	0	+	+	0	+	0	+	0	2+	2+
9	rr	0	0	0	+	+	0	0	0	+	+	+	0	+	0	+	0	+	0	0	0	+	0	+	0	0	0	+	0	2+	2+
10	rr	0	0	0	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	0	0	+	+	+	+	+	0	+	0	2+	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	+	+	0	+	+	0	+	2+	NT	NT
Last wash SC1																													0	2+	2+
Last wash SC2																													0	2+	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Additional History

Additional laboratory results from the patient show a reticulocyte count of 22.3%, lactate dehydrogenase (LDH) 877 U/L, and total bilirubin 4.1 mg/dL with direct bilirubin 1.1 mg/dL. The haptoglobin is less than 8 mg/dL. The patient had been transfused with two units of RBCs during the transplant surgery. Based on the patient’s antibody results, the blood bank medical director reviews the pretransplant results as below.

ABO/Rh/Antibody Screen

Patient

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A1 cells	B cells
0	0	3+	4+	4+
Extended Rh phenotype: C– c+ E+ e+				
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	0			
Reaction scale = 0 (no reaction) to 4+ (strong reaction)				

Liver Donor Sample

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A1 cells	B cells
0	0	0	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	2+			
SC2	2+			
Reaction scale = 0 (no reaction) to 4+ (strong reaction)				

Donor Units Transfused During Transplant Surgery

Donor unit	ABO/Rh	Extended Rh phenotype
Donor unit #1	O-positive	D+ C+ c + E+ e+/R ₁ R ₂
Donor unit #2	O-positive	D+ C+ c– E–/R ₁ R ₁

Gel Panel (Liver Donor Sample)

Cell #	Rh-hr	Rh-hr										Kell				Duffy		Kidd		Lewis		MNS			P		Lutheran		Test results
		D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	1+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	1+
3	R ₂ R ₂	+	+	0	+	0	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+	+ ^S	0	+	1+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	+	+	+	+	+	0	+	1+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	0	+	0	0	+	0	+	+	0	+	1+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	0	+	0	+	+	+	+ ^w	0	+	0	0
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+ ^S	0	+	0	0
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	0	+	+	+	+ ^S	0	+	0	0
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	0	+	0	+	0	0	0	+	0
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+ ^w	0	+	0	0
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+	+	0	+	1+
Patient cell			0	0	2+																								0

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Questions

1. What do the patient's pre- and posttransplant ABO/Rh type and screen results show?
2. What is your interpretation of the patient's posttransplant antibody panel, direct antiglobulin test (DAT), and eluate results? What is the likely source of the identified antibodies?
3. How do you correlate the immunohematology findings with the clinical summary in this case?
4. How would you treat this patient's condition in light of the antibody findings?

Answers

1. **What do the patient's pre- and posttransplant ABO/Rh type and screen results show?** The results show that the patient is group O, Rh-positive (refer to Chap. 1, question 1 answer for information about forward and reverse ABO typing) and that the antibody screen was negative prior to the transplant but has become positive in the posttransplant sample.
2. **What is your interpretation of the patient's posttransplant antibody panel, DAT, and eluate results? What is the likely source of the identified antibodies?** Anti-D and anti-C antibodies are evident on the gel panel. These antibodies are coating the RBCs from the patient (which includes patient's native RBCs plus transfused donor RBCs) as evidenced by a positive immunoglobulin (Ig)G DAT and findings of the antibodies on the eluate panel (note that while the patient's RBCs are C-antigen negative, the transfused donor RBCs are positive for the C antigen). One possible explanation for the presence of these antibodies is that the patient has been alloimmunized through prior transfusion or pregnancy with anti-D being an antibody against partial D antigen since the patient types as Rh(D)-positive (see Chap. 21, question 4 answer for discussion on partial D). A second possibility is that passenger lymphocytes from the liver graft are producing the anti-D and anti-C antibodies (note that the organ donor's RBCs are rr based on the Rh phenotype results on the gel panel). To distinguish between these two possibilities, molecular testing for the Rh blood group system, to identify a partial D genotype in particular, can be performed (see Chap. 30 for more information on RBC genotyping). However, since the antibody panel from the organ donor sample also showed antibodies to the Rh(D) and Rh(C) antigens (i.e., the liver donor was immunized to the D and C antigens prior to death), passenger lymphocyte syndrome (PLS) is more likely. One final possibility is that the apparent antibody combination of anti-D and anti-C may actually be anti-G antibody from the passenger lymphocytes (see Chap. 11 for more information about anti-G), though clinically, this distinction is not important under the circumstances in this case (i.e., Rh immune globulin [RhIg] administration is not a consideration for this patient).

3. **How do you correlate the immunohematology findings with the clinical summary in this case?** The Rh antibodies appear to be related to RBC hemolysis. In the absence of bleeding, dropping hemoglobin despite the reticulocytosis is consistent with hemolysis. The markedly decreased haptoglobin level suggests that there is an intravascular component to the hemolysis. In general, it is more likely that anti-D and anti-C antibodies cause extravascular hemolysis. These findings in the setting of a liver donor with Rh(D) and Rh(C) sensitization are consistent with PLS. PLS occurs when donor lymphocytes within a solid organ allograft produce antibodies against RBC antigens expressed on the recipient's red cells. It usually appears 14–21 days after transplant. Most commonly, it occurs when there is a minor ABO mismatch. For example, when a liver graft from a group B donor is transplanted into a group AB recipient, the passenger lymphocytes produce anti-A antibodies which then bind to the A antigen on the group AB recipient's RBCs. The greatest risk occurs in the combination of a group O donor with a group A recipient [1]. The anti-A/B IgG isotype found in group O patients is more potent than either anti-A or anti-B. Less commonly, the donor can be immunized against irregular blood group antigens, for example, the Rh (D, C/c, E/e) and Kidd blood group systems [2]. The clinical significance can vary from just having antibody coating the RBCs to fulminant hemolysis as seen in this case. The risk factors for PLS include the dose of lymphocytes within the allograft (with heart and lung organ transplants having the greatest risk followed by liver and kidney grafts) as well as the immune status of the recipient [2].
4. **How would you treat this patient's condition in light of the antibody findings?** PLS is generally self-limited with good outcomes though graft failure and death have also been known to occur [2]. There are a few case reports and series describing successful treatment, but no standard treatment regimen is used. In the presence of brisk hemolysis, anemia can be treated with RBC transfusion of fully crossmatch-compatible red cells lacking the antigens targeted by the red cell alloantibodies (i.e., group O-negative, C-negative [rr] cells in this case). RBC exchange transfusion can be considered if a significant number of native red cells are still circulating [3]. Alternatively, therapeutic plasma exchange (TPE) can be performed to remove the circulating antibodies [3]. Intravenous immunoglobulin (IVIG) has also been used successfully. Corticosteroids or other immunosuppressants are also often administered to decrease antibody production [2]. If antibody production is still resistant, rituximab (monoclonal anti-CD20/B-cell marker antibody) may be used. The antibodies should disappear in approximately 3 months but may persist longer.

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3. Cserti-Gazdewich CM, Waddell TK, Singer LG, Chaparro C, Pendergrast JM, Hawes J, et al. Passenger lymphocyte syndrome with or without immune hemolytic anemia in all Rh-positive recipients of lungs from rhesus alloimmunized donors: three new cases and a review of the literature. *Transfus Med Rev*. 2009;23(2):134–45.

Recommended Reading

Cserti-Gazdewich CM, Waddell TK, Singer LG, Chaparro C, Pendergrast JM, Hawes J, et al. Passenger lymphocyte syndrome with or without immune hemolytic anemia in all Rh-positive recipients of lungs from rhesus alloimmunized donors: three new cases and a review of the literature. *Transfus Med Rev*. 2009;23(2):134–45.

Chapter 33

In the Clouds



Clinical History

A 10-year-old boy presents with a history of multiple recurrent infections including pneumonia, lymphadenitis, and osteomyelitis since the age of 2 months. The patient currently has a pulmonary mycetoma that is refractory to medical treatment and is scheduled for surgical resection. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank with a request for two units of red blood cells (RBCs) for surgery.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	4+	0	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	2+			
SC2	2+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel Panel

Cell #		Rh-hr		Rh-hr										Kell						Duffy			Kidd		Lewis		MNS			P		Lutheran		Test results
		D	C	E	c	e	f	c ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b						
1	R _{1w} R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	1+				
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	0	+	2+					
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	0	+	0	+	+	s ₊	0	+	2+				
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	+	0	0	0	+	+	+	+	+	0	+	2+				
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	1+	+	0	0	+	0	+	+	0	+	1+				
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	0	+	+	+	0	+	+	+	+	w ₊	0	+	2+					
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+	s ₊	0	+	2+				
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	0	+	+	+	s ₊	0	+	2+					
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	0	0	+	2+					
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	+	w ₊	0	+	2+					
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	+	+	+	0	+	2+				
Patient cell																														0				

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Further Immunohematology Workup

As a result of the findings on the above antibody panel which are suggestive of an antibody to a highfrequency antigen requiring specialized workup, the patient's sample is sent to the immunohematology reference laboratory for further testing as below.

Antigen Phenotyping Record (Tube Testing with Rare Sera)

Rh-hr										Kell				Duffy		Kidd		Lewis		MNS		P	Lutheran				
	D	C	E	c	e	f	c ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P _I	Lu ^a	Lu ^b
Patient cell	+	+	0	+	+	0	0	0	0	m	0	m	0	m	0	0	+	+	0	+	0	0	+	+	+	0	+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); *m* microscopic agglutination

Selected Cell Panel (with Rare Donor Red Cells)

		Rh-hr							Kell					Duffy		Kidd	Lewis		MNS			P	Lutheran	Test result					
Cell #	Donor	D	C	E	c	e	f	c ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P _I	Lu ^a	Lu ^b	AHG gel
1	Kx-donor*	+	+	0	0	+	NT	NT	NT	0	+	0	NT	NT	NT	+	+	0	+	0	0	0	+	+	+	+	NT	NT	1+
2	⁰ donor	+	+	0	0	+	NT	NT	NT	0	0	0	0	0	0	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	3+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); *Kx- negative donor cell, K⁰ Kell null

Questions

1. What antibodies did you identify?
2. What information does phenotyping of the patient provide in this case, and how does it relate to the patient's history of recurrent infections, including refractory fungal infection?
3. When viewing the peripheral blood smear from this patient, what unusual RBC morphology might you see?
4. What other clinical manifestations may be seen in this patient and what enzyme is typically elevated?
5. What recommendations would you make for this patient for RBC transfusion during surgery?

Answers

1. **What antibodies did you identify?** From the selected cell panel, one can identify anti-Kx given the weaker reaction with the Kx-negative donor versus the K⁰ donor cells, the latter having increased expression of Kx antigen. However, persons with McLeod phenotype, who lack both Km and Kx antigens, also develop anti-Km. In particular, transfused McLeod persons without chronic granulomatous disease (CGD) develop anti-Km alone, while those with CGD develop anti-Kx plus anti-Km [1].
2. **What information does phenotyping of the patient provide in this case, and how does it relate to the patient's history of recurrent infections, including refractory fungal infection?** This patient has X-linked CGD, a primary immunodeficiency caused by mutations in the *gp91^{phox}* (*CYBB*) gene, leading to impaired phagocytic killing and repeated infections with bacterial and fungal pathogens. A minority of patients with X-linked CGD have large deletions that also include the neighboring *XK* gene which encodes the Xk protein and hence the Kx antigen, which is usually present on all red cells. Absence of the Kx antigen causes the rare McLeod syndrome (150 reported cases), characterized by weakened expression of Kell system antigens, acanthocytosis, compensated hemolysis, and neurologic deterioration. This patient's RBCs were negative for K, Kp^a, and Js^a antigens and only microscopically reactive with anti-k, anti-Kp^b, and anti-Js^b. These results are suggestive of the McLeod phenotype [2, 3].
3. **When viewing the peripheral blood smear from this patient, what unusual RBC morphology might you see?** As noted above, acanthocytosis may be prominent.
4. **What other clinical manifestations may be seen in this patient?** As noted above, patients with McLeod syndrome may also exhibit neurologic symptoms including muscle weakness, seizure, and chorea (involuntary muscle movements). Typically, creatine kinase is elevated.

5. **What recommendations would you make for this patient for RBC transfusion during surgery?** The anti-Kx and anti-Km antibodies will react with all red cells that express either Kell system antigens or the Kx antigen, rendering all units incompatible except those from another person with the McLeod phenotype. It should be noted that Kell null (K^0) red cells react strongly with anti-Kx due to increased apparent expression of the Kx antigen and would be inappropriate for such a patient. As much as possible, transfusion should be avoided in this patient. Possible transfusion strategies include donation of autologous blood before surgery, location of blood from a rare donor program, or directed donation of blood from a sibling with the McLeod phenotype. In an emergent situation, the patient could potentially be given O-negative or type-specific (A-positive) RBCs in the event of significant blood loss, with careful monitoring for a delayed hemolytic transfusion reaction. Finally, blood conservation strategies, including intraoperative cell salvage and acute normovolemic hemodilution, may be considered.

References

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Chapter 34

How “Dara” You!



Clinical History

A 73-year-old man with immunoglobulin (Ig)G kappa multiple myeloma is referred for outpatient transfusion due to severe anemia with hemoglobin (Hgb) of 6.6 g/dL. The patient has a history of red blood cell (RBC) transfusion at your hospital 2 years ago at which time the antibody screen was negative. An ethylenediaminetetraacetic acid (EDTA) anticoagulant sample is submitted to the blood bank for type and screen along with a request for two units of irradiated RBCs.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	1+			
SC2	W+			

Reaction scale = 0 (no reaction) to 4 + (strong reaction); W weak

Gel Panel

Cell #	Rh-hr	Rh-hr										Kell				Duffy		Kidd		Lewis		MNS			P		Lutheran		Test results
		D	C	E	c	e	f	c ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	0	0	+	1+
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	+	0	+	+	S ₊	0	+	2+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	+	+	+	+	+	0	+	2+
5	r' r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+	+	+	0	+	W+
6	r'' r	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	0	0	0	+	0	+	+	+	W ₊	0	+	1+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	+	+	+	+	0	0	+	+	+	S ₊	0	+	1+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	0	S ₊	0	+	2+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	0	0	+	+	0	+	0	+	1+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	+	W ₊	0	+	1+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	+	+	0	+	1+
Patient cell																													0

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Additional History

Further history is obtained in regard to the positive findings in the antibody screen and panel. The patient is noted to be on medications including daratumumab, lenalidomide, and dexamethasone for treatment of the multiple myeloma. Based on this history, additional blood bank testing is performed. Meanwhile, the outpatient RBC transfusions are rescheduled for the following day, pending outcome of the additional testing and crossmatch of RBC units.

Gel and Dithiothreitol (DTT) Panel

Cell #	Rh-hr	Kell										Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results							
		D	C	E	c	e	f	c ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a		Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG Gel	DTT
1	R ₀ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	1+	0
2	R ₁ R ₁	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	1+	0	
3	R ₂ R ₂	+	0	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	0	0	0	+	0	+	+	+	0	+	2+	0
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	+	0	+	2+	0
5	r ¹ r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	0	+	W+	0
6	r ¹ r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+	0	+	1+	0
7	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	0	0	+	0	+	+	+	0	+	W+	0
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	0	+	+	+	0	+	2+	0
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	0	+	0	+	1+	0	
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+	+	+	0	+	1+	0
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	+	+	+	0	+	1+	0
Patient cell																													0	NT	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Further History

Two units of group O, Rh-positive, K-antigen-negative RBCs are crossmatched as below and transfused without any complications.

PEG Crossmatch

PEG crossmatch results		
	AHG	CC
Donor unit #1 (O-positive)	1+	NT
Donor unit #2 (O-positive)	W+	NT

Questions

1. What is the apparent nature of the patient’s antibody findings based on the clinical history and the antibody panels?
2. How does the dithiothreitol (DTT) panel help to resolve the nature of the antibody results? What is the significance of the K-antigen-negative phenotype of the crossmatched RBC units in this case? What alternative testing could be done to resolve these antibody findings?
3. Why are the two donor RBC units incompatible?
4. What additional testing would you advise at this time to make future transfusions of this patient safer against the risk of hemolytic transfusion reactions due to presence of undetected alloantibodies resulting from daratumumab interference?

Answers

1. **What is the apparent nature of the patient’s antibody findings based on the clinical history and antibody panels?** It is apparent that the cause of the antibody reactions is related to daratumumab medication that the patient is taking as part of his treatment for multiple myeloma. Daratumumab is an IgG1 kappa human monoclonal antibody that targets human CD38 cell-surface receptor which is highly expressed on myeloma cells [1]. It is indicated for treatment of previously treated multiple myeloma. However, since RBCs also weakly express CD38 antigen, daratumumab binds to RBCs causing panreactivity in the antibody screen and panel indirect antiglobulin tests (IAT) which may persist for up to 6 months after the last dose of the medication [1, 2]. Although in this case, the

autocontrol result is nonreactive, positive autocontrol and IgG direct antiglobulin (DAT) results may also occur [1]. Note that ABO and Rh typing are not affected by daratumumab. Interestingly, and perhaps of concern for immunohematologists, other agents that may interfere with routine blood bank testing are already in development, such as anti-CD47, a humanized monoclonal antibody that may be effective against hematological and solid tumors [3]. Meanwhile, the efficacy of daratumumab is being investigated for use in other conditions such as advanced amyloidosis and pediatric T-cell acute lymphoblastic leukemia [4, 5].

2. **How does the DTT panel help to resolve the nature of the antibody results? What is the significance of the K-antigen-negative phenotype of the cross-matched RBC units in this case? What alternative testing could be done to resolve these antibody findings?** DTT is a reducing agent that breaks disulfide bonds and denatures CD38 on RBCs, preventing daratumumab from binding and interfering with antibody testing. Thus, the DTT panel in this case is nonreactive in all cells, revealing that there are no unexpected alloantibodies identified. One must be cautious, however, when working with DTT since some RBC blood group antigens such as Cartwright (YT), Dombrock (DO), Kell, Landsteiner-Weiner (LW), Lutheran (Lu), and Knops are notably destroyed so that antibodies to these blood group antigens cannot be reliably detected [1]. Therefore, it is prudent to crossmatch K-antigen-negative RBCs if the patient is known to lack K antigen or if the K-antigen phenotype is unknown (recall that the frequency of K antigen is less than 10% so that the majority of people will lack K antigen [refer to Table of RBC Antigen Frequencies in the front section of this workbook]) to prevent potential hemolytic transfusion reactions from occurring in the case that anti-K antibodies are present but not detected because of DTT treatment of the reagent panel cells. Testing with trypsin is an alternative method that disrupts the extracellular domain of CD38, allowing for detection of unexpected RBC alloantibodies [1]. Like DTT, though, trypsin also removes K antigen [6]. However, it should be noted that most hospital blood banks do not use trypsin or DTT for their routine testing, and so, they are unable to perform the workup of patients receiving daratumumab on-site.
3. **Why are the two donor RBC units incompatible?** The two crossmatched O-positive, K-negative RBC units are incompatible because of interference with daratumumab (note that they are only incompatible at the antihuman globulin [AHG] phase of crossmatching but would be compatible by immediate-spin [IS] crossmatch).
4. **What additional testing would you advise at this time to make future transfusions of this patient safer against the risk of hemolytic transfusion reactions due to presence of undetected alloantibodies resulting from daratumumab interference?** Genotyping can be used to obtain a predicted phenotype of RBC antigens so that phenotype-matched blood (i.e., matched for clinically significant antigens, typically C, c, E, e, K, Jk^a, Jk^b, Fy^a, Fy^b, S, and s) can be crossmatched to avoid hemolytic transfusion reactions. Refer to Chap. 30 for more information about RBC antigen genotyping.

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Chapter 35

Polly Wants a Cracker!



Clinical History

A 3-year-old girl is admitted to the emergency department with fever of 101.6°F accompanied by poor feeding over the prior 3 days and a persistent cough with dyspnea. Chest x-ray reveals left lower lobe pneumonia, and blood cultures are positive for *Streptococcus pneumoniae*. The child was born prematurely at 34 weeks by vaginal delivery and weighed 2.5 kg (current weight 12.4 kg). Laboratory values are as follows: white blood cells (WBC) 20.0 K/ μ L, hemoglobin (Hgb) 7.2 g/dL, hematocrit (Hct) 24%, platelet count 62 K/ μ L, lactate dehydrogenase (LDH) 800 U/L, prothrombin time (PT) 17.2 s, activated partial thromboplastin time (aPTT) 48.5 s, total bilirubin 5.0 mg/dL, and creatinine 4.2 mg/dL. An ethylenediaminetetraacetic acid (EDTA) anticoagulant sample is submitted to the blood bank for type and screen.

ABO/Rh/Antibody Screen

ABO/Rh (tube method)				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	0	4+	4+
Antibody screen (tube LISS method)				
	37 °C	AHG	CC	
SC1	0	0	2+	
SC2	0	0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

The patient is admitted to the pediatric intensive care unit (PICU). Based on the laboratory findings of anemia, thrombocytopenia, and renal failure along with schistocytes (3 per high-power field) seen on the peripheral blood smear, the pediatric clinical team highly suspects pneumococcal-associated hemolytic uremic syndrome (pHUS). Further testing is performed in a reference laboratory which shows that the patient’s red blood cells (RBCs) are incompatible by immediate-spin (IS) crossmatch with five out of five donor plasma samples tested but compatible with cord blood serum. The direct antiglobulin test (polyspecific DAT) is negative. In addition, the patient’s RBCs are tested against a panel of lectins as per the table below. Meanwhile, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) level is reported as 72% (normal range, greater than 60%).

Lectin	Reaction: patient RBCs
<i>Arachis hypogaea</i> ^a	+
<i>Glycine soja</i> ^b	+
<i>Salvia horminum</i>	–
<i>Salvia sclarea</i>	–

^aPeanut lectin
^bSoybean lectin

Further History

The patient’s treatment in the PICU includes mechanical ventilation, continuous peritoneal dialysis, and vancomycin and cephalosporin antibiotics for sepsis. A request is received in the blood bank for RBC and plasma transfusion at a dose of 10 mL/kg (120 mL) for each product. The pediatric clinical team also inquires about the possibility of performing therapeutic plasma exchange (TPE).

Questions

1. What is the difference between thrombotic thrombocytopenic purpura (TTP) and atypical hemolytic uremic syndrome (aHUS)?
2. What phenomenon is being shown by the lectin panel, and what mechanisms may be involved in causing this phenomenon? How does this phenomenon relate to the patient’s diagnosis of pHUS?

3. In light of this patient's condition and the request for RBC and plasma transfusion, what unique consideration should be taken into account?
4. What are your recommendations for TPE in this patient, as per the request from the pediatric clinical team?

Answers

1. **What is the difference between thrombotic thrombocytopenic purpura (TTP) and atypical hemolytic uremic syndrome (aHUS)?** Although TTP and aHUS were formerly considered to be a part of the same disease spectrum, mainly differentiated by the degree of renal failure in the latter, the two entities are now understood to have different underlying causes. The cause of TTP is known to be deficiency of ADAMTS13, usually through the development of inhibitors (acquired TTP) though congenital deficiency (Upshaw–Schulman syndrome) has also been described. Deficiency of ADAMTS13, a metalloprotease that cleaves von Willebrand factor (VWF) multimers, leads to circulation of unusually large VWF multimers causing thrombocytopenia through formation of platelet microthrombi and hemolytic anemia. Daily TPE with plasma replacement to remove the inhibitor and replace ADAMTS13 is considered as the mainstay therapy to correct thrombocytopenia and the hemolytic anemia. Steroid therapy is also considered to be beneficial to suppress the inhibitor. Resistant cases may need to be treated with the addition of intravenous immunoglobulin (IVIG) and/or rituximab (monoclonal anti-CD20/B- cell marker antibody) therapy. Meanwhile, the classification of hemolytic uremic syndrome (HUS) has become even more diversified; classically, cases were divided into diarrhea-positive HUS associated with Shiga toxin *Escherichia coli* (STEC) and non-diarrhea aHUS. However, this classification has broadened to include aHUS associated with complement factor mutations and inhibitors (complement factors H and I) as well as pHUS. As a result, newer treatments have been developed for aHUS, especially eculizumab, a monoclonal antibody that acts as a terminal C5 complement pathway inhibitor that is also indicated for treatment of patients with paroxysmal nocturnal hemoglobinuria and generalized myasthenia gravis [1, 2]. Eculizumab, however, is not indicated for STEC hemolytic uremic syndrome.
2. **What phenomenon is being shown by the lectin panel, and what mechanisms may be involved in causing this phenomenon? How does this phenomenon relate to the patient's diagnosis of pHUS?** The lectin panel is showing polyagglutination via T-activation. This phenomenon occurs when the so-called cryptantigen (TF antigen, named after the codiscoverers Thomsen and Friedenreich) underneath the cell surface of blood cells (including RBCs, WBCs,

and platelets) becomes exposed, usually via the action of bacterial neuraminidase during acute infections [2]. As a result, T-activated RBCs become agglutinable by all human adult plasma or serum which naturally contains immunoglobulin (Ig)M antibodies to T antigen (however, neonatal [cord blood] serum does not contain these antibodies and so does not agglutinate T-activated RBCs, as noted in this case). Other forms of acquired and inherited polyagglutination, such as Th, Tk, Tn, and Tx, exist and may be the result of myelodysplastic syndrome or acute leukemia or congenital conditions such as hereditary erythroblastic multinuclearity with positive acidified serum test (HEMPAS, a genetic anemia associated with incomplete RBC membrane antigen glycosylation and polyagglutination) [3–5]. Also of interest to students of immunohematology, the acquired B phenotype is a type of polyagglutination. Lectins (as shown in the panel below; recall that lectins are plant-protein extracts that bind to carbohydrates on cell membranes) are used to differentiate the various types of polyagglutination. Polyagglutination relates to pHUS in that T-activation may occur as a result of infection with *Streptococcus pneumoniae*. Note that T antigen is also found in renal endothelium which may play a role in the development of aHUS in pneumococcal infection [3].

Lectin	T	Th	Tk	Tn	Tx
<i>Arachis hypogaea</i> ^a	+	+	+	–	+
<i>Glycine soja</i> ^b	+	–	–	+	–
<i>Salvia horminum</i>	–	–	–	+	–
<i>Salvia sclarea</i>	–	–	–	+	–
<i>Vicia cretica</i>	+	+	–	–	–
<i>Dolichos biflorus</i>	–	–	–	+	–
<i>Medicago disciformis</i>	+	+	–	–	–
<i>Bandeiraea simplicifolia II</i>	–	–	+	–	–

^aPeanut lectin
^bSoybean lectin

3. **In light of this patient’s condition and the request for RBC and plasma transfusion, what unique consideration should be taken into account?** There have been reports of severe and even fatal intravascular hemolysis associated with transfusion of blood products containing antibodies to T antigen in T-activated infants [3]. However, it is not entirely clear whether such hemolysis was directly caused by transfusion. Nevertheless, some have recommended transfusion protocols to reduce exposure to T antibodies in such individuals, including the use of washed or plasma-reduced RBCs and platelets and low-titer anti-T plasma (though, admittedly, such plasma is not readily available) [3]. Incidentally, autoagglutination or hemolysis does not occur in vivo in T-activated individuals, apparently because of either T antibody adsorption or immune tolerance/paralysis [3].

4. **What are your recommendations for TPE in this patient, as per the request from the pediatric clinical team?** While there is only limited experience of TPE for treatment of pHUS, and it is listed by the American Society of Apheresis (ASFA) as a category III indication (optimum role of apheresis not established), the benefits of TPE may include removal of antibodies to T antigen and clearance of circulating bacterial neuraminidase [6]. The ASFA TPE guidelines also recommend use of albumin fluid replacement to avoid passive transfer of anti-T from plasma [6]. Nevertheless, TPE in a small child requires special considerations and preparation, including the consideration of extracorporeal volume relative to the patient's whole blood volume and the need for priming of the apheresis device bowl to minimize excessive hypovolemia during the procedure as well as issues with hypothermia and citrate-induced hypocalcemia (citrate is often used as the anticoagulant of choice during TPE). Vascular access can also be challenging in these pediatric cases.

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Chapter 36

Hot and Cold



Clinical History

A 5-year-old boy presents to the emergency department (ED) with 2 days of fever (temperature 100.7 °F), fatigue, jaundice, and dark urine. The parents note that the child had symptoms of a viral upper respiratory illness 2 weeks before presentation and that he was previously healthy with no history of blood transfusion. In the ED, the child weighs 21.3 kg, and laboratory workup reveals severe anemia with hemoglobin (Hgb) 5.4 g/dL, elevated total bilirubin 5.1 mg/dL, aspartate aminotransferase (AST) 357 U/L (normal range, 8–60 U/L), and lactate dehydrogenase (LDH) 8126 U/L. Serum haptoglobin is undetectable. Urinalysis results are as follows: color, dark; red blood cells (RBCs), 0–3 per high-power field; and urine Hgb, 3+ positive. Notably, the onset of symptoms (fever, fatigue, jaundice, dark urine) was associated with eating a bowl of ice cream. An ethylenediaminetetraacetic acid (EDTA) anticoagulant type and screen sample is submitted to the blood bank along with a request for RBC transfusion.

ABO/Rh/Antibody Screen

Patient

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

The child receives the RBC transfusion (approximately 14 mL/kg, 300 mL total); however, the Hgb falls to 4.1 g/dL within 30 h of presentation. As a result of the apparent hemolytic anemia in the child, further workup is performed in the blood bank:

DAT profile					
Polyspecific:	Polyspecific: 3+	Anti-IgG:	0	Anti-C ₃ d:	3+

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

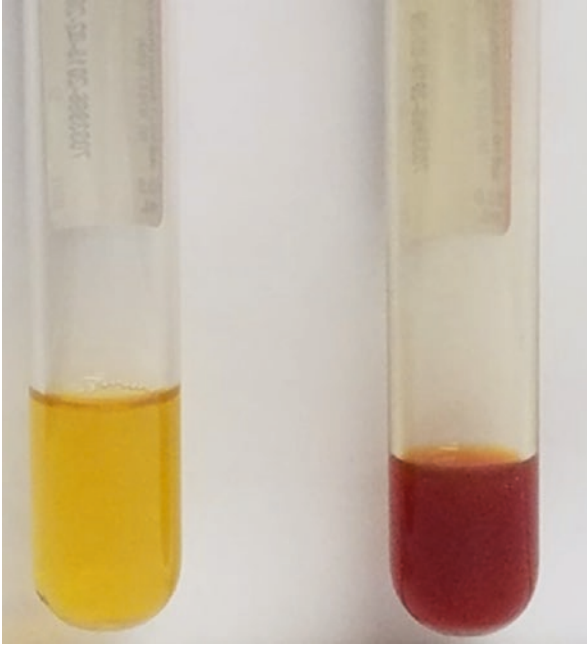
Cold antibody screen (tube LISS method)				
	IS	RT	15 °C	37 °C
SC1	0	W+	2+	0
SC2	0	W+	2+	0

Further Immunohematology Workup

Due to the clinical history, a fresh whole blood sample is collected into a red-top tube (no anticoagulant), kept at 37 °C until clotted, and the serum is sent to the immunohematology reference laboratory for special testing as follows.

Donath–Landsteiner test			
Incubation with group O, P+ RBCs	Visual inspection for hemolysis		
	Patient serum	Patient + normal serum mix	Normal serum
Ice bath (4 °C) followed by 37 °C	3+	3+	0
Ice bath (4 °C) only	0	0	0
37 °C only	0	0	0

Reaction scale = 0 (no reaction) to 4+ (strong reaction)
Donath–Landsteiner Test: Patient’s serum after incubation with group O, P-positive RBCs at 4 °C for 30 min followed by 37 °C incubation for 60 min is seen on the right compared with normal serum. Photo credit: Ajit Bharne, Department of Pathology/Blood Bank, Mount Sinai West, New York, NY



Further History

The patient is kept warm, is treated with oral steroids (prednisone 1 mg/kg/day), and receives another transfusion of RBCs. After 2 days, he is transferred to the pediatric intensive care unit (PICU) due to hypotension (80/54 mmHg) and progressive acute renal impairment. Treatment with intravenous (IV) methylprednisolone (1 mg/kg/day) is added, and an additional RBC transfusion is given. Laboratory and clinical indicators of hemolytic anemia begin to improve on hospital day #5; the LDH steadily decreases with increase of the reticulocyte count and stabilization of the Hgb over the next 4 days. The child is discharged on hospital day #10.

Questions

1. What is the apparent cause of the child's anemia? What is the name of the causative antibody and what unusual property does it have?
2. How does the serologic testing help you to establish the diagnosis in this case? What precaution must be taken when handling and submitting the blood specimen to the testing laboratory for serologic workup?
3. What options are there for clinical management of this child?

Answers

- 1. What is the apparent cause of the child's anemia? What is the name of the causative antibody and what unusual property does it have?** This clinical presentation is typical of paroxysmal cold hemoglobinuria (PCH). PCH is an uncommon autoimmune hemolytic anemia, historically associated with syphilis (the Donath–Landsteiner [D–L] antibody, the classical underlying cause of PCH, in fact, was first observed as a cross-reacting antibody to an antigen on *Treponema pallidum*), but is now most often seen in children after a viral illness [1]. PCH is reported to account for 5–10% of pediatric autoimmune hemolytic anemias (AIHA) [1, 2]. Rare cases of PCH in adults have been described in association with chronic lymphocytic leukemia (CLL) and lymphoma (PCH accounts for less than 1% of AIHA in adults) [1]. The childhood form is typically acute, self-limiting, and steroid-responsive, but occasional cases may be severe, as in this case. The D–L antibody is an immunoglobulin (Ig)G antibody with specificity for the P antigen. The antibody is referred to as a “biphasic hemolysin” because of the fact that RBC destruction is primarily due to antibody binding and complement fixation at cold temperature with subsequent intravascular hemolysis at warm temperature. However, there is also a component of extravascular hemolysis with reticuloendothelial system clearance of RBCs coated with complement (C₃b and C₄b). Peripheral blood smears may reveal RBC rosetting around neutrophils and erythrophagocytosis [3].
- 2. How does the serologic testing help you to establish the diagnosis? What precaution must be taken when handling and submitting the blood specimen to the testing laboratory for the serologic workup?** The direct antiglobulin test (DAT) is typically negative for IgG and positive for complement (C3), as in this case. During routine testing, the antibody screen (indirect antiglobulin test [IAT]) is negative; this is because antihuman globulin (AHG) screening reagents do not detect complement, and the IgG antibody dissociates from the RBC surface at 37 °C. Therefore, the autoantibody is not readily identified using plasma or eluted plasma. The D–L test involves collecting fresh whole blood from the patient and maintaining the sample at 37 °C until clot formation to prevent autoadsorption of autoanti-P antibodies at low temperatures and then separating the serum. Group O, P-positive RBCs are then incubated with three specimens: (1) the patient's serum, (2) a mixture of the patient's and normal serum (as a source of fresh complement), and (3) normal serum. The samples are incubated at 4 °C for 30 min followed by 37 °C incubation for 60 min, with visible hemolysis indicative of a positive reaction. If the D–L antibody is present, samples #1 and #2 should be positive. As negative controls, the three samples may be replicated at testing conditions in which the temperature is maintained at 4 °C and also at 37 °C throughout testing. However, to limit the volume of blood draws in an anemic, small child, sample #3 and the negative controls may be omitted. A modified antibody screen may also be performed by incubating control RBCs

with the patient's serum and then washing with ice-cold saline solution to avoid dissociating the D–L antibody from the RBCs. Monoclonal IgG antiserum is then added.

3. **What options are there for clinical management of this child?** The prognosis of PCH is frequently very good. Treatment is typically aimed at keeping the child warm until the episode resolves; steroid therapy is often administered, although the evidence supporting its efficacy is limited. In this case, due to the severe anemia and renal impairment, critical care support and RBC transfusions were indicated. P-antigen-negative blood is not usually necessary (note that RBCs lacking the offending P-antigen group [i.e., p RBCs] are very rare [only 1 in 200,000] and difficult to obtain) [4]; patients generally respond to routine allogeneic RBCs. Case reports have also suggested that rituximab (monoclonal anti-CD20/B-cell marker antibody) may be of benefit in the treatment of refractory PCH in adults [5]. Rarely, chronic or recurrent forms of PCH occur [6].

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Chapter 37

Mix and Match



Clinical History

A 50-year-old woman with acute myeloid leukemia (AML) receives a hematopoietic stem cell transplant (HSCT) from a human leukocyte antigen (HLA)-matched (10/10 antigens) related donor (peripheral blood stem cells [PBSC] collected via apheresis with stimulation by granulocyte colony-stimulating factor [G-CSF] and containing at least 5×10^6 CD34+ cells/kg) post-induction chemotherapy and myeloablative conditioning. The patient and donor ABO/Rh types and antibody screens are shown below (ethylenediaminetetraacetic acid [EDTA] anticoagulant samples).

Patient/Recipient Pre-HSCT ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	3+	4+	4+
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	0	0	2+	
SC2	0	0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

HSCT Donor ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	3+	0	4+
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	0	0	2+	
SC2	0	0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

Despite recovery of white blood cells (absolute neutrophil count [ANC] greater than 1.5 K/ μ L) and platelets (greater than 50 K/ μ L) within 30 days post-HSCT, the patient/recipient’s hemoglobin (Hgb) level has remained less than 7.0 g/dL with reticulocytopenia (less than 1.0% reticulocytes) noted, requiring ongoing support with multiple transfusions of red blood cells (RBCs). A type and screen sample (EDTA anticoagulant) is submitted to the blood bank at post-HSCT day #50 along with a request for transfusion of two units of RBCs.

Patient/Recipient Post-HSCT ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	3+	2+	4+
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	0	0	2+	
SC2	0	0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Further History

The RBC transfusions (two units) are completed without incident on post-HSCT day #50. Complete engraftment is seen at post-HSCT day #80, following a course of therapeutic plasma exchange (TPE), with a rise and stabilization of the Hgb level to greater than 10 g/dL.

Patient/Recipient Post-HSCT Engraftment ABO/Rh/Antibody Screen

<i>ABO/Rh (tube method)</i>				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	3+	0	4+
<i>Antibody screen (tube LISS method)</i>				
	37 °C	AHG	CC	
SC1	0	0	2+	
SC2	0	0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Questions

1. What is the patient/recipient's versus the HSCT donor's ABO type?
2. In terms of ABO types, what kind of HSCT is this? What is the direction of incompatibility?
3. What problem may arise as a result of the incompatibility, and what complication has occurred (as seen at post-HSCT day #50) in the patient/recipient as a consequence of the incompatibility present in this case? What manipulation of the HSCT product can be done to minimize such complications?
4. What would be the direction of incompatibility if the donor's ABO type was group O while the recipient's was group A? What complications may arise from this type of incompatibility? What HSCT product manipulation should be done in this type of incompatibility?
5. What would be the direction of incompatibility if the donor's ABO type was group A while the recipient's was group B? What complications may arise from this type of incompatibility? What HSCT product manipulation should be done in this type of incompatibility?
6. How would you manage transfusion in this patient/recipient pre- and immediately post-HSCT transplant?
7. How would you manage transfusion in this patient/recipient after engraftment with the donor's blood type?
8. In consideration that the patient/recipient in this case received an HSCT product that was collected from peripheral blood (PBSC) via apheresis, what sources are available for collection of hematopoietic stem cells (HSCs), and what are the advantages and disadvantages of each?

Answers

1. **What is the patient/recipient's versus the HSCT donor's ABO/Rh type?** The patient/recipient's type is group O-positive while the HSCT donor's type is group A-positive. Refer to Chap. 1, question 1 answer for information on ABO/Rh forward and reverse typing.
2. **In terms of ABO types, what kind of HSCT is this? What is the direction of incompatibility?** This HSCT is one of mismatched ABO types, known as an ABO-incompatible HSCT. Given that the patient/recipient is group O type while the donor is group A, this is a major incompatibility; that is, recipient anti-A isoantibodies are directed against donor group A antigens (conversely, in minor incompatibility, donor antibodies are directed against recipient antigens, such as in the case of a group O donor's anti-A targeting recipient group A antigen). It is important to understand that HLA matching takes precedence over ABO matching when it comes to HSCT; however, the opposite is generally true for solid organ transplants (i.e., ABO matching generally takes precedence over HLA matching in solid organ transplant due to risk of hyperacute rejection).
3. **What problem may arise as a result of the incompatibility, and what complication has occurred in the patient/recipient (as seen at post-HSCT day #50) as a consequence of the incompatibility present in this case? What manipulation of the HSCT product can be done to minimize such complications?** The group A RBCs in the HSCT product may be hemolyzed by the anti-A isoantibodies still circulating in the patient/recipient's plasma. In addition, circulating anti-A isoantibodies can lead to delayed RBC engraftment of the donor group A RBCs and, if severe, can lead to pure red cell aplasia (PRCA) [1]. In fact, PRCA has occurred in this patient/recipient, given that the patient's Hgb has remained low with reticulocytopenia and presence of anti-A isoagglutinin still in the patient/recipient's reverse type on day #50 post-HSCT. Group O patients receiving group A, B, or AB grafts are at risk of major incompatibility and delayed engraftment. Desensitization methods, such as TPE, may be necessary in order to treat such cases; the American Society for Apheresis (ASFA) lists major ABO-incompatible HSCT as a category II level indication (disorders for which apheresis is accepted as second-line therapy, either as a standalone treatment or in conjunction with other modes of treatment) for TPE [2]. When major incompatibility exists, as in this case, the HSCT product should undergo RBC reduction in order to reduce the volume of incompatible RBCs in the product. Each laboratory must establish the maximum threshold volume of incompatible RBCs (e.g., 30 mL), above which the HSCT product will require RBC reduction; RBC reduction may also be necessary when the recipient's isoantibody titer is high (e.g., anti-A titer [immunoglobulin (Ig)G or IgM] greater than 32).
4. **What would be the direction of incompatibility if the donor's ABO type was group O while the recipient's was group A? What complications may arise from this type of incompatibility? What HSCT product manipulation should be done in this type of incompatibility?** In minor incompatibility,

donor antibodies are directed against recipient antigens. Minor incompatibility occurs when a group A, B, or AB recipient receives an HSCT from a group O donor. There is risk of hemolysis due to the anti-A or anti-B isoantibodies in the donor unit [1]. Plasma reduction of the HSCT product should be performed in these cases. RBC exchange transfusion may also be considered for the patient/recipient; ASFA lists minor ABO-incompatible HSCT as category III level indication (optimum role of apheresis not established) for RBC exchange (though the level of evidence for this recommendation is weak, grade 2C). A few weeks after minor-incompatible HSCT, there is a risk of passenger lymphocyte syndrome (PLS) occurring whereby the donor lymphocytes produce anti-A and/or anti-B against the recipient's native RBCs resulting in hemolysis (see Chap. 32 for more information on PLS). The patient should be monitored for 5–15 days after transplantation with serial Hgb, lactate dehydrogenase (LDH), and bilirubin levels as well as visual inspection for hemolysis. A direct antiglobulin test (DAT) and eluate may also be performed to demonstrate the incompatible iso-hemagglutinin coating the native red cells.

5. **What would be the direction of incompatibility if the donor's ABO type was group A while the recipient's was group B? What complications may arise from this type of incompatibility? What HSCT product manipulation should be done in this type of incompatibility?** A group A donor and group B recipient, or group B donor and group A recipient, are considered a bidirectional (major and minor) incompatibility. The donor HSCT product has incompatible RBCs and isoantibodies that are incompatible with the recipient's RBCs and plasma. In these cases, there are also the delayed risks associated with major and minor incompatibility. Delayed RBC engraftment, PRCA, and PLS may all occur. Both RBC- and plasma-reduction product manipulations should be performed for bidirectional incompatibility. TPE should also be considered.
6. **How would you manage transfusion in this patient/recipient pre- and immediately post-HSCT transplant?** Pretransplant, the patient/recipient should receive ABO/Rh-compatible RBCs for the patient's original blood type. Therefore, the patient could receive group O-positive RBCs and any plasma type (A, B, AB, or O). Immediately following the HSCT, the patient should be transfused with blood products that are compatible with both the donor and the recipient. Therefore, the patient must receive group O RBCs and type A or AB plasma.
7. **How would you manage transfusion in this patient/recipient after engraftment with the donor's blood type?** After engraftment, when the RBCs and the isohemagglutinins are of the donor type, then the patient/recipient can receive ABO/Rh-compatible RBCs matched to the donor type. In this case, the recipient should receive group A RBCs and type A plasma. Group O RBCs and type AB plasma are also compatible and could be transfused, though given inventory considerations and desire to conserve these product types, they would be secondary choices.
8. **In consideration that the patient/recipient in this case received an HSCT product that was collected from peripheral blood (PBSC) via apheresis, what sources are available for collection of HSCs, and what are the advan-**

tages and disadvantages of each? HSCs, which are identified by the CD34 marker (i.e., CD34+ cells), can be collected from three sources. Traditionally, they were obtained through harvesting of bone marrow (marrow stem cells [MSC]); however, as this is a highly invasive procedure performed under general anesthesia, collection from peripheral blood (PBSC) using apheresis devices has become the preferred method in many cases. Yet a newer concept is harvesting the stem cells from umbilical cord blood (UCBSC) during delivery of the baby (the first UCBSC transplant was performed in 1988) [3]. The table below outlines advantages and disadvantages of each source [4].

HSC source	Advantages	Disadvantages
MSC	Rich source of HSCs does not require use of mobilization agent (G-CSF); lower risk of GVHD	Invasive harvesting procedure performed under general anesthesia; possible higher graft failure risk versus PBSC
PBSC	Less invasive harvesting procedure using apheresis collection device; possible lower risk of graft failure versus MSC; survival equivalent to MSC	Requires use of mobilization agent (often, G-CSF, which may have side effects such as bone pain, headache, and flu-like symptoms); greater T-cell content with increased risk of GVHD (particularly, chronic GVHD)
UCBSC	Noninvasive harvesting; lower risk of chronic GVHD versus MSC and PBSC; public cord banking expands readily available donor pool of HSC products (particularly in cases in which a matched-sibling HSC donor is not available)	Higher risk of graft failure and delayed hematopoietic recovery in adults related to lower HSC dose that may require double UCBSC transplants (which have higher risk of acute GVHD versus single UCBSC transplants); controversial use of private over public banks to store UCBSC products which are rarely needed but expensive for families to maintain

G-CSF granulocyte colony-stimulating factor, *GVHD* graft-versus-host disease, *HSC* hematopoietic stem cells, *MSC* marrow stem cells, *PBSC* peripheral blood stem cells, *UCBSC* umbilical cord blood stem cells.

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1. Daniel-Johnson J, Schwartz J. How do I approach ABO-incompatible hematopoietic progenitor cell transplantation? *Transfusion*. 2011;51(6):1143–9.
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Chapter 38
Golden Grans



Clinical History

A 34-year-old woman with Philadelphia chromosome-positive pre-B-cell acute lymphocytic leukemia who received a matched-related-donor myeloablative hematopoietic stem cell transplant (HSCT) 3 days ago is admitted to the hospital with worsening abdominal exam and a new fever (temperature 39.5 °C). The patient is diagnosed with gram-negative typhlitis (neutropenic enterocolitis) and started on antibiotic therapy; however, the patient’s condition does not improve. Alternate-day granulocyte transfusions over 10–14 days are requested by the patient’s hematologist to help treat the bacterial infection in the setting of profound neutropenia (white blood cells [WBCs] less than 1.0 K/ μ L) until HSCT engraftment occurs. An ethylenediaminetetraacetic acid (EDTA) anticoagulant sample is submitted to the blood bank for type and screen.

ABO/Rh/Antibody Screen

ABO/Rh (tube method)				
Patient RBCs (forward type)			Patient plasma (reverse type)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
Antibody screen (tube LISS method)				
	37 °C	AHG	CC	
SC1	0	0	2+	
SC2	0	0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

The patient, who is known to be cytomegalovirus (CMV) antibody positive, receives the first two granulocyte transfusions (irradiated granulocytes from group O-positive donors) without adverse effect and has remained afebrile over the past 48 hours. However, during the third granulocyte transfusion, after infusion of 175 mL of product, the patient develops rigors and severe abdominal pain. The granulocyte transfusion is immediately stopped, and 500 mL of normal saline, acetaminophen, and hydromorphone are given. A posttransfusion sample is submitted to the blood bank for workup of the suspected transfusion reaction.

Test Results: Posttransfusion Sample

Clerical check			
Patient: O-positive		Donor granulocyte unit: O-positive	
Visual check: no hemolysis			
	Polyspecific	Anti-IgG	Anti-C ₃ d
DAT (pre-sample)	0	NT	NT
DAT (post-sample)	0	NT	NT

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Further History

The transfusion reaction is further investigated as below.

Time	Patient’s vital signs				
	Temperature °C	Pulse Beats/minute	Blood pressure mmHg	Respiratory rate Breaths/minute	Oxygen saturation
Pretransfusion (15:00)	36.9	128	139/78	14	100%
16:00	36.3	124	146/93	16	100%
17:00	37.0	135	133/84	17	100%
Suspected reaction (17:30)	38.0	155	179/101	19	100%
Posttransfusion (17:45)	37.6	168	134/71	26	100%
18:00	37.5	165	115/52	26	100%

Patient laboratory values	Pretransfusion	Posttransfusion
White blood cells (WBC)	0.09 K/ μ L	0.14 K/ μ L
Hemoglobin (Hgb)	8.4 g/dL	8.1 g/dL
Hematocrit (Hct)	24.6%	24.2%
Platelets	18 K/ μ L	13 K/ μ L

Patient/donor	ABO/Rh	Human leukocyte antigens (HLA)
Patient/HSCT recipient	O-positive	
HSCT donor	O-positive	
Granulocyte donor (third unit)	O-positive	A02,03; B15:01,40:01

Patient/recipient HLA class 1 antibody screen	
Panel-reactive antibody (PRA) 38%	HLA antibodies Anti-A2,68,69/weak anti-B45, 44, 48, 57

Questions

1. Is the request for granulocyte transfusion appropriate?
2. Based on the clinical presentation and the findings, how would you classify the transfusion reaction that occurred in this patient?
3. What is the most likely cause of the patient's transfusion reaction?
4. What are other risks of granulocyte transfusion?

Answers

1. **Are the granulocyte transfusions appropriate for this patient?** Although the literature on granulocyte transfusion to treat refractory infections is limited to low-quality evidence, it is generally agreed that granulocyte transfusion may be considered if (1) the patient is profoundly neutropenic, (2) the patient has failed appropriate antimicrobial therapy, and (3) marrow recovery is expected. Since this patient has a very low absolute neutrophil count (ANC, calculated by multiplying the total WBCs by the percent of neutrophils in the differential; ANC typical normal range is 1.5–8.0 K/ μ L), has refractory infection, and is expected to have marrow recovery when the HSCT engrafts, she meets the criteria for granulocyte transfusions.
2. **Based on the clinical presentation and the findings, how would you classify the transfusion reaction that occurred in this patient?** The granulocyte product contains up to 50 mL of red blood cells (RBCs), but this product was compatible with both the recipient's native ABO type and the HSCT donor's ABO type

(note that as a result of these RBCs in granulocyte concentrates, the concentrates must be ABO compatible and crossmatched just like RBC products). There is no evidence of hemolysis in the posttransfusion sample, and there is no drop in the patient's hemoglobin. This transfusion reaction is best classified as a febrile non-hemolytic transfusion reaction (FNHTR). It meets the National Healthcare Safety Network (NHSN) Hemovigilance Module definition of FNHTR: the reaction occurs during or within 4 h of cessation of transfusion, and either fever (oral temperature greater than or equal to 38 °C/100.4 °F and a change of at least 1 °C/1.8 °F from the pretransfusion value) or chills/rigors are present.

3. **What is the most likely cause of the patient's transfusion reaction?** Febrile transfusion reactions and pulmonary complications including transfusion-related acute lung injury (TRALI) are well-recognized complications of granulocyte transfusion and are more likely to occur in patients with pre-existing granulocyte-reactive (HLA or human neutrophil antigen [HNA]) antibodies. This patient was found to have HLA antibodies directed against antigens present on the transfused donor leukocytes (anti-HLA A2). It has been shown that transfused granulocytes migrate to sites of infection; this may sometimes lead to localized pain at the site or increased radiologic appearance of infiltrates.
4. **What are other risks of granulocyte transfusion?** Because granulocyte concentrates must be transfused within 24 h of collection, products may be released before the results of infectious disease testing are complete; therefore, granulocyte transfusion poses a greater theoretical infectious risk compared with other blood products, particularly due to intracellular pathogens such as CMV (note that the patient in this case is CMV-antibody positive, though, which reduces such risk of CMV transmission; also note that some donor collection centers may prescreen their granulocyte donors for infectious disease up to 30 days before the collection to reduce the risk of transmissible disease. Unlike other cellular blood products (RBCs and platelets), granulocytes must never be leukoreduced (this point is actually rather intuitive since why would one remove the very cells that one is trying to give to the patient?). Due to the presence of viable lymphocytes in the granulocyte product, patients are also at risk of transfusion-associated graft-versus-host disease (TA-GVHD), a rare but highly fatal complication that results when transfused T-lymphocytes engraft, proliferate, and attack host tissue antigens in a recipient who is unable to reject the allogeneic cells, either due to immune compromise or HLA similarity to the donor. To prevent this complication, granulocyte concentrates must be irradiated before transfusion. Meanwhile, there are also increased risks associated with donation of granulocytes, particularly in donors who are mobilized with corticosteroids or granulocyte colony-stimulating factor (G-CSF, though such use is not approved by the US Food and Drug Administration [FDA]) in order to improve collection yield which is expected to be at least 1×10^{10} granulocytes per collection (note that since granulocyte transfusions are typically ordered as a set of five to seven daily to alternate-day transfusions for a given patient, multiple donors are necessary to support the patient). Donors who receive G-CSF may experience bone pain, headache, and flu-like symptoms [1].

Reference

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Recommended Reading

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Chapter 39

I “Pitty” Thee



Clinical History

A 74-year-old man is admitted to hospital for workup of gastrointestinal (GI) bleeding; he is scheduled for colonoscopy the following day. An ethylenediaminetetraacetic acid (EDTA) anticoagulant sample is submitted to the blood bank for type and screen.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4 + (strong reaction); W weak

Additional History

In preparation for the procedure, the night float intern reviews the patient's laboratory values as follows: white blood cells (WBC) 5.2 K/ μ L, hemoglobin (Hgb) 7.8 g/dL, hematocrit (Hct) 26%, and platelet count 162 K/ μ L. However, the intern notices that the coagulation test results (prothrombin time [PT] and activated partial thromboplastin time [aPTT]) are still pending, though the samples were submitted several

hours ago. Upon contacting the hematology laboratory, the intern learns that the coagulation blue-top (citrate) sample, drawn by a nurse, was insufficient for testing (the tube was only half-filled). Therefore, the intern collects a new sample from the patient (conveniently drawing the sample from the patient’s intravenous [IV] access line), ensuring that the blue-top tube is completely filled, and brings it to the hematology laboratory for stat testing. Thirty minutes later, the intern receives an alert value notification from the laboratory supervisor that the aPTT result is 96 s. Meanwhile, the PT is reported as 15.0 s. The intern, acting on sign out instructions from the daytime medicine resident to “correct abnormal hematology parameters for planned endoscopy in the AM,” orders two units of fresh frozen plasma (FFP) which are transfused over the next 3 h. A planned red blood cell (RBC) transfusion is canceled after the patient develops respiratory distress with bibasilar pulmonary rales noted on chest auscultation and elevated blood pressure (increase from 130/86 to 146/100 mmHg) which improves with IV furosemide diuretic and supplemental oxygen by nasal cannula. Repeat laboratory test results from the morning specimen draw, collected by the phlebotomist, show that the aPTT has “corrected” to 36 s and the PT is 12.0 s. The colonoscopy is performed later in the morning without complication.

Questions

1. In consideration of the above clinical scenario, what is the likely cause of the patient’s elevated aPTT laboratory value? What additional laboratory testing could have been done to confirm the cause of the elevated aPTT?
2. How did the abnormal aPTT contribute to the intern’s decision to transfuse FFP? Was plasma transfusion indicated? If not, what measures might the transfusion service implement to prevent such transfusions in the future?
3. What untoward event did the patient experience as a result of the transfusion of plasma?

Answers

1. **In consideration of the above clinical scenario, what is the likely cause of the patient’s elevated aPTT laboratory value? What additional laboratory testing could have been done to confirm the cause of the elevated aPTT?** It is likely that the sample drawn by the night float intern from the patient’s IV access line was contaminated with heparin resulting in a disproportionately elevated aPTT (note that the PT is only slightly elevated). However, since an elevated aPTT can be the result of a number of different causes, some of which are associated with clotting (like a lupus anticoagulant, a type of antiphospholipid antibody) while others may cause bleeding (like an acquired factor VIII inhibi-

tor), it may be important to perform additional testing to elicit the underlying cause; naturally, it is equally important to obtain history of thrombosis and bleeding from the patient, including family history. In this case, the easiest step for the laboratory to take is to repeat the aPTT test on the same sample using heparinase; if there is indeed heparin contamination of the sample, the aPTT result will correct due to neutralization of the heparin. Alternatively, a new sample may be collected by proper venipuncture to avoid heparin contamination and submitted to the laboratory. Other laboratory tests that may be useful in the workup of bleeding or clotting disorders are included in the table below which outlines some of the common coagulation tests performed in the hematology laboratory (note that the tests are listed for reference only and that not all will be necessary in the case of an elevated aPTT).

Test name	Test principle ^a	Test result interpretation
Activated partial thromboplastin time (aPTT)	Patient's citrated plasma is incubated with phospholipid/partial thromboplastin and calcium mixture in the presence of kaolin or silica activator; time to clot formation is measured	Prolonged (typical normal range, 32–40 s): 1. Congenital or acquired deficiencies of factors (F)VIII, IX, XI, XII 2. Inhibitors to FVIII, FIX, FXI 3. Lupus anticoagulant 4. Disseminated intravascular coagulation (DIC) 5. Heparin 6. Direct thrombin inhibitor (DTI, dabigatran etexilate); variable with FXa inhibitors (apixaban, edoxaban, and rivaroxaban)
Prothrombin time (PT)	Patient's citrated plasma is incubated with thromboplastin (mixture of phospholipid and tissue factor); time to clot formation is measured; note that the international normalized ratio (INR) is a calculation derived from the patient's PT, pooled normal PT, and the international sensitivity index of the thromboplastin reagent	Prolonged (typical normal range, 11–14 s): 1. Coumadin (warfarin) therapy 2. Vitamin K deficiency 3. DIC 4. Congenital and acquired deficiencies of fibrinogen, thrombin (FII), FV, FVII, FX 5. Inhibitors to FII, FV, FX (including DTI and FXa inhibitors)
Mixing study	Performed when aPTT and/or PT are prolonged; patient's plasma is mixed with pooled normal plasma (1:1 ratio) and incubated at 37 °C for various times (0 min, 30 min, 1 h); aPTT and/or PT tests are run after mixing and incubation to look for correction	aPTT or PT corrects by mixing with pooled normal plasma if prolongation is due to a deficiency of one or more factors. In the presence of an inhibitor, prolonged aPTT or PT will not correct or only partially correct

Test name	Test principle ^a	Test result interpretation
Thrombin time	Thrombin (FII) is incubated with patient's plasma; time to clot formation is measured	Prolonged (typical normal range: < 17 s): 1. Heparin 2. Congenital and acquired fibrinogen deficiency or dysfibrinogenemia 3. DIC 4. Fibrinolysis, increased fibrin degradation products (FDP) 5. DTI
Fibrinogen assay	Clauss method (dilute thrombin time; functional assay): Thrombin is added to dilute patient plasma; time to clot formation is measured and fibrinogen level is determined from a standard curve Antigenic method: Enzyme-linked immunosorbent assay (ELISA); immunologic measure of fibrinogen	Low fibrinogen level (typical normal range, 150–400 mg/dL): 1. DIC 2. Fibrinolysis 3. Congenital afibrinogenemia or hypofibrinogenemia 4. Post-plasmapheresis with albumin fluid replacement Note that fibrinogen is an acute phase reactant that is typically elevated in inflammatory states as well as in pregnancy
FDP (also known as fibrin split products) and D-dimer (D-D) assay; note that D-D is a specific type of FDP that results from plasmin degradation of fibrin and not from fibrinogen degradation	Latex agglutination method: Patient's plasma is mixed with latex beads coated with monoclonal anti-FDP or anti-D-D antibodies. Visible agglutination occurs if FDP or D-D is present in the patient's plasma and may be quantitated by testing serial dilutions of the patient's plasma	Elevated (typical normal range FDP < 10 mcg/mL; D-D < 0.5 mcg/mL): 1. DIC 2. Fibrinolysis 3. Thrombolytic therapy 4. Deep venous thrombosis and pulmonary embolism 5. Postsurgery 6. Severe liver disease (reduced clearance of FDP)
Reptilase time	Performed when thrombin time is prolonged; patient's plasma is incubated with Reptilase-R (thrombin-like enzyme that is not inhibited by heparin or FDP); time to clot formation is measured	Prolonged (typical normal range, <24 s) in hypofibrinogenemia or dysfibrinogenemia; normal in presence of heparin
Dilute Russell viper venom test (DRVVT)	Patient's plasma (spun to remove platelets which are a source of phospholipid) is incubated with snake venom (which directly activates FX in the presence of phospholipid, thrombin, and calcium); time to clot formation is measured	Prolonged (typical normal range, 29–42 s) in the presence of a lupus anticoagulant (antiphospholipid antibody); not affected by deficiency of or inhibitors to FVII, FVIII, FIX, FXI, and FXII. The presence of lupus anticoagulant may be confirmed by neutralization with additional phospholipid to correct DRVVT

^aNote that more than one test method may be available for some of the listed tests

2. **How did the abnormal aPTT contribute to the intern's decision to transfuse FFP? Was plasma transfusion indicated? If not, what measures might the transfusion service implement to prevent such transfusions in the future?** It is apparent from the clinical case scenario that the intern acted upon a laboratory alert notification in a “knee-jerk” fashion to correct the aPTT based on a vague communication from the senior resident or perhaps the GI physician planning the colonoscopy. Clearly, sample heparin contamination does not warrant plasma transfusion. Unfortunately, stamping out such unnecessary and potentially harmful transfusions can be quite vexing. Institution of evidence-based transfusion guidelines, clinical education on transfusion practices, monitoring use of blood products, and implementation of prospective audits (i.e., screening blood product requests prior to transfusion) are all recommended strategies to reduce inappropriate transfusions.
3. **What untoward event did the patient experience as a result of the transfusion of plasma?** This patient evidently suffered from an episode of transfusion-associated circulatory overload (TACO) as a result of transfusion of the two units of plasma totaling a volume of 500–700 mL over a 3-h period. TACO is a common problem associated with fluid administration, including blood products, and is particularly prone in frail elderly patients or in patients with renal failure or a history of congestive heart failure. It is also a leading cause of transfusion-related fatalities along with transfusion-related acute lung injury (TRALI), ABO incompatible transfusions, and bacterial septic reactions. Fortunately, this episode of TACO was not severe and responded promptly to the administration of furosemide diuretic. Nevertheless, this event was entirely avoidable: pity the patient, indeed!

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Chapter 40

All Washed Up



Clinical History

A 22-year-old man with a history of homozygous factor (F)V deficiency (Owren's disease) is admitted for an elective right knee arthroscopy. Coagulation laboratory studies show prothrombin time (PT) 18.6 s and activated partial thromboplastin time (aPTT) 55.1 s. A type and screen (ethylenediaminetetraacetic acid [EDTA] anticoagulant) sample is submitted to the blood bank along with a request for three units of fresh frozen plasma (FFP) for preoperative transfusion.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	4+	0	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

Less than 10 min into the transfusion of the first unit of FFP, the patient develops hives and shortness of breath, which progresses to laryngeal bronchospasm and oxygen desaturation with hypotension (blood pressure drop from 130/80 to

100/60 mmHg). A rapid response emergency is called and the patient is given diphenhydramine and hydrocortisone, but he continues to rapidly decompensate and requires intubation. A posttransfusion sample (EDTA anticoagulant) is submitted to the blood bank for workup of the transfusion reaction.

Clerical check

Patient: A-positive

Donor unit: A-positive

Visual check: no hemolysis

	Polyspecific	Anti-IgG	Anti-C ₃ d
DAT (pre-sample)	0	NT	NT
DAT (post-sample)	0	NT	NT

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Further History

The elective arthroscopic procedure is postponed pending a hematology consultation to evaluate the patient and make recommendations regarding management of the patient's FV deficiency as well as management in the case that the patient requires transfusion of red blood cells (RBCs).

Questions

1. Considering the clinical scenario, how would you classify the transfusion reaction that occurred in this patient? What condition is associated with this type of reaction?
2. What additional testing would you perform to determine if this patient requires special blood product modifications?
3. In case the patient needs RBC and/or platelet transfusion, would you recommend use of washed RBCs and/or platelets? How does washing affect the blood product?
4. What alternatives are there to the use of washed RBCs or platelets? Are there any alternatives to FFP?
5. Regarding treatment of the patient's FV deficiency, aside from FFP transfusion, what other treatment options may be considered?

Answers

1. **Considering the clinical scenario, how would you classify the transfusion reaction that occurred in this patient? What condition is associated with this type of reaction?** The patient ostensibly experienced an anaphylactic hypersen-

sitivity reaction. Classically, immunoglobulin (Ig)A deficiency has been associated with anaphylactic transfusion reactions. However, this is poorly supported in the literature. The cause of most allergic transfusion reactions is due to donor, component product, or recipient factors, and the majority of anaphylactic reactions to blood products are unrelated to IgA (the overall incidence of anaphylactic transfusion reactions is 1:20,000 to 1:50,000 transfusions and mostly involve plasma or platelet transfusions) [1, 2]. However, it is important to note that selective IgA deficiency can be found in approximately 1 in 700 persons of European ancestry, though fortunately, only a small percent of IgA-deficient persons make anti-IgA [1]. Haptoglobin deficiency with associated haptoglobin antibodies has also been rarely identified as a cause of anaphylactic transfusion reactions, particularly in Japan [3].

2. **What additional testing would you perform to determine if this patient requires special blood product modifications?** Additional testing (i.e., testing for IgA deficiency) is not usually necessary as most severe allergic reactions are isolated events and are rarely associated with an identifiable trigger [2]. Careful monitoring, premedication with antihistamine and steroids, and supportive care are the recommended course for future transfusion needs. If the patient has repeated, severe anaphylactic reactions, testing for IgA deficiency and anti-IgA antibodies is an option.
3. **In case the patient needs RBC and/or platelet transfusion, would you recommend use of washed RBCs and/or platelets? How does washing affect the blood product?** Washing a red cell product reduces the cell mass of the product, as some cells are inevitably lost in the process [4]. More significant is the impact of washing on platelets, which not only results in a small loss of product but also in an increase in spontaneous platelet activation of up to 50%; thus, the washed platelets are less effective for in vivo hemostasis [5]. As a result of the loss of quality as well as storage time of washed cellular products (washed RBCs typically have a shelf life of 24 h, while washed platelets expire after 4 h; this is due to the open washing system which increases the risk of bacterial contamination), alternatives to washed products should be considered (see question 4 answer below).
4. **What alternatives are there to the use of washed RBCs or platelets? Are there any alternatives to FFP?** Volume reduction of cellular blood products has also been shown to be effective in reducing allergic reactions and may be tried before resorting to washed products. Another option currently available for platelet transfusion is the use of apheresis platelets stored in additive solution (platelet additive solution or PAS); these are platelets in which approximately 70% of the plasma has been replaced by a nutrient solution (refer to Chap. 16, question 4 answer). Meanwhile, there is some evidence that solvent-detergent plasma (pooled plasma that is treated by solvent/detergent agents to inactivate lipid-enveloped viruses such as hepatitis B and C viruses [HBV, HCV] and human immunodeficiency virus [HIV]) may have a lower rate of allergic reactions than FFP [6]. Finally, transfusion of blood components collected from IgA-deficient donors should be reserved for patients who are proven to have absent IgA along with broad class-specific anti-IgA antibodies [2].

5. **Regarding treatment of the patient's FV deficiency, aside from FFP transfusion, what other treatment options may be considered?** Currently, there is no Food and Drug Administration (FDA)-approved FV concentrate available in the United States; however, platelet transfusion might be a better alternative than FFP for treatment of FV deficiency. This is because, although FV is a labile factor (along with FVIII) that is lost during product storage, platelets have a protected source of FV in their α -granules which has greater procoagulant potential and is released at the site of vascular injury [7].

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Recommended Reading

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Chapter 41

The Red Pee



Clinical History

A 55-year-old man with B-cell lymphoma and history of a warm autoantibody and a cold autoanti-I antibody reactive at 4 °C but no alloantibodies is admitted to the hospital for workup of fever of unknown origin. The patient has received red blood cell (RBC) and platelet transfusions in the hospital in the past but none in the prior 3 months. An ethylenediaminetetraacetic acid (EDTA) anticoagulant sample is submitted to the blood bank for type and screen along with a request for crossmatch of one irradiated RBC unit; the patient's hemoglobin (Hgb) is noted to be 7.0 g/dL at the time of the request.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	3+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	1+			
SC2	1+			

Reaction scale = 0 (no reaction) to 4 + (strong reaction); W weak

Gel Panel

		Rh-rh						Kell				Duffy				Kidd		Lewis		MNS			P	Lutheran	Test results					
Cell #	Rh-rh	D	C	E	c	e	f	c _w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel	
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	1+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	0	+	+	+	0	0	0	0	+	+	+	0	0	0	+	1+
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	0	0	0	+	+	0	0	0	+	0	+	S ₊	0	0	+	1+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	0	0	0	0	0	0	0	0	+	+	+	+	0	0	+	1+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	0	0	+	+	0	+	+	0	0	+	0	+	+	0	+	1+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	0	+	0	0	0	+	0	+	+	+	+	w ₊	0	0	+	1+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	0	+	0	+	+	+	0	0	+	0	+	S ₊	0	0	+	1+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	0	+	+	+	0	+	+	0	+	+	0	S ₊	0	0	+	1+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	0	+	0	+	+	0	0	+	0	+	0	0	0	0	+	1+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	0	+	0	+	+	+	0	+	+	+	+	w ₊	0	0	+	1+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	1+
Patient cell																														1+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

DAT profile			
Polyspecific:	1+	Anti-IgG:	1+

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Anti-C₃d:

0

Acid Eluate Panel

		Rh-hr								Kell				Duffy		Kidd		Lewis		MNS				P	Lutheran		Test results: IAT/tube					
Cell #	Rh-hr	D	C	E	c	e	f	c ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P _I	Lu ^a	Lu ^b	AHG	CC		
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	1+	NT	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	0	+	1+	NT	NT	
3	R ₂ R ₂	+	+	0	+	0	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+	S ₊	0	+	1+	NT	NT	
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	0	+	1+	NT	NT	
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	0	+	1+	NT	NT	
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	0	0	+	+	+	+	W ₊	0	+	1+	NT	NT	
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+	S ₊	0	+	1+	NT	NT	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+	+	S ₊	0	+	1+	NT	NT	
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	0	+	0	0	+	0	0	+	1+	NT	NT	
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	0	+	+	+	+	W ₊	0	+	1+	NT	NT	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	0	+	+	0	+	0	+	1+	NT	NT
Last wash SC1																													0	2+		
Last wash SC2																													0	2+		

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Antigen phenotype results	
	D+ C+ E- c+ e+ K- Fy ^a + Fy ^b - Jk ^a + Jk ^b - S- s+ M+ N+ P+ Le ^a - Le ^b +

Autoadsorption Panel

		Rh-hr						Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube						
Cell #	Rh-hr	D	C	E	c	e	f	c ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC
1	R _{1w} R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	0	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	0	0	+	+	0	2+
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	0	+	s ₊	+	+	0	2+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	0	0	+	+	+	+	+	+	0	2+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+	+	0	2+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	+	+	w ₊	0	+	0	2+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	s ₊	+	+	0	2+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	+	s ₊	0	+	0	2+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	2+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	0	+	+	+	+	w ₊	0	+	0	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	+	0	+	+	0	2+
Patient cell																													0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Additional History

Based on the above test results, the patient is transfused one unit of irradiated O-positive RBCs, weakly incompatible (“least incompatible”) by antihuman globulin (AHG) crossmatch. Thirty minutes after completion of the RBC unit, the nurse reports that the patient has passed dark-red urine; a posttransfusion (EDTA anticoagulant) sample is sent to the blood bank for workup of a transfusion reaction. A urine sample sent to the laboratory shows the following test results: color, dark; RBCs, 1–3 per high-power field; urine Hgb, positive; and urine myoglobin, negative.

Test Results: Posttransfusion Sample

<i>Clerical check</i>			
Patient: O-positive		Donor unit: O-positive	
<i>Visual check: sample is grossly hemolyzed</i>			
	Polyspecific	Anti-IgG	Anti-C ₃ d
DAT (pre-sample)	1+	1+	0
DAT (post-sample)	1+	1+	0

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Acid Eluate Panel (Posttransfusion Sample)

Rh-hr											Kell				Duffy		Kidd		Lewis		MNS			P	Lutheran		Test results: IAT/tube				
Cell #	Rh-hr	D	C	E	c	e	f	c ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG	CC	
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	1+	NT
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	0	+	+	1+	NT
4	R ₀ r	+	0	0	+	+	0	0	+	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	+	+	0	+	1+	NT
5	r'r	0	+	0	+	+	0	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	0	0	+	+	0	+	1+	NT
6	r''r	0	0	+	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	+	+	+	w ₊	0	+	1+	NT
7	rr	0	0	0	+	+	0	0	0	+	+	0	+	0	+	0	+	+	+	0	0	0	+	0	+	+	s ₊	0	+	1+	NT
8	rr	0	0	0	+	+	0	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	0	+	s ₊	0	+	1+	NT
9	rr	0	0	0	+	+	0	0	0	0	+	+	+	0	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	1+	NT
10	rr	0	0	0	+	+	0	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	w ₊	0	+	1+	NT
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	0	0	+	0	+	1+	NT
Last wash SC1																														0	2+
Last wash SC2																														0	2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Alloadsorption Panel (Posttransfusion Sample, Adsorbed using Patient Phenotype-Matched R₁R₁ Cells*)

		Rh-hr						Kell						Duffy		Kidd		Lewis		MNS				P	Lutheran		Test results: IAT/tube					
		D	C	E	c	e	f	c ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N		S	s	P ₁	Lr ^a	Lr ^b	AHG	CC	
Cell #	Rh-hr	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	+	+	+	0	2+
1	R _{1w} R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	0	0	+	+	0	2+	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	+	+	+	+	0	0	+	+	0	2+	
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	0	0	0	+	0	+	+	s	+	+	+	0	2+	
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	0	0	+	+	+	+	+	+	+	+	0	2+	
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	0	2+	
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	+	+	w	+	+	+	0	2+	
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	+	+	+	+	s	+	+	+	0	2+	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	+	s	+	+	+	0	2+	
9	rr	0	0	0	+	+	+	0	0	0	+	+	0	+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	2+	
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	w	+	+	+	0	2+	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	0	0	+	0	+	+	+	+	+	+	+	+	0	2+	
Patient cell																														0	2+	

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak
*Ficin-treated R₁R₁ (K- Jk^b-) cells

Further Investigation

Upon further investigation, the involved nurse noted that she was instructed by the patient's doctor to place the RBC unit in a warm water bath for 30 min prior to transfusion because of the patient's history of a "cold agglutinin." The nurse followed the instructions but did not measure the temperature of the water bath. The nurse stated that the RBC unit felt "very warm" prior to transfusion.

Questions

1. What is the apparent cause of the patient's passing of dark-red urine based on history and testing?
2. By what mechanism did the patient's passing of dark-red urine probably occur? Besides the probable mechanism in this case, what other mechanisms could lead to such an event?
3. How could the event have been prevented in this particular case?

Answers

1. **What is the apparent cause of the patient's passing of dark-red urine based on history and testing?** The patient passed dark-red urine 30 min after RBC transfusion; the initial test results revealed hemolysis as evidenced by hemoglobinuria, low haptoglobin with elevated lactate dehydrogenase (LDH), and a grossly hemolyzed posttransfusion sample. However, although the patient has warm autoantibodies present in his plasma and coating his RBCs (i.e., a positive immunoglobulin [Ig]G direct antiglobulin test [DAT]) as well as a history of cold-autoanti-I (cold autoantibody), it is evident that the acute hemolysis does not likely have an immune basis since the former would only be a cause of extravascular hemolysis (i.e., would not result in hemoglobinuria) while the latter is only reactive at cold temperature and would not cause *in vivo* hemolysis. Additionally, based on the alloadsorption (performed using patient phenotype-matched RBCs due to the recent transfusion; recall that autoadsorption should not be performed in the case that the patient has received transfusion in the prior 3 months as this could lead to a false-negative result; see Chap. 7, question 3 answer), no new clinically significant alloantibodies were identified. Thus, hemolysis appears to be nonimmune in nature (i.e., nonimmune acute hemolysis). Also, it should be noted that myoglobinuria, which can mimic hemoglobinuria but is caused by extensive skeletal muscle damage as in crush injury or in extensive surgery (e.g., spinal surgery) [1], is ruled out in this case by a negative urine myoglobin result as well as lack of any clinical reason for muscle injury.

2. **By what mechanism did the patient's passing of dark-red urine probably occur? Besides the probable mechanism in this case, what other mechanisms could lead to such an event?** Based on information obtained from the involved nurse, it is likely that the RBC unit was exposed to a high temperature that caused damage to the RBCs (i.e., thermal hemolysis). Thermal hemolysis may occur when the RBCs are exposed to very warm temperatures (i.e., greater than 42 °C) or very cold temperatures (in fact, when freezing RBCs for long-term storage [up to 10 years at -80 °C or below], a cryoprotectant agent, typically glycerol, must be used to prevent hemolysis). Besides temperature-induced hemolysis, RBCs may be damaged by traumatic handling (i.e., mechanical hemolysis) of the unit such as attempting to increase the rate of transfusion by squeezing or placing a pressure-band around the bag or by use of a rapid pressure infuser. Additionally, mechanical hemolysis may occur if the blood is infused through a very small-bore needle. A third mechanism that may damage RBCs is osmotic hemolysis; this occurs when RBCs are exposed to hypotonic solutions such as half-normal (0.45%) saline or 5% dextrose in water (D5W, although there is some evidence that 10% dextrose in water [D10W], a hypertonic solution, may not adversely affect RBCs) [2]. For this reason, RBC units should not be mixed with any solution other than normal (0.9%) saline or an equivalent US Food and Drug Administration (FDA)-approved solution (such as Normosol-R® [Hospira, Inc., Lake Forest, IL, USA] or Plasma-Lyte A® [Baxter Healthcare Corp., Deerfield, IL, USA]). In rare instances, it is worth noting that nonimmune hemolysis may be related to an underlying condition of the patient, such as glucose-6-phosphate dehydrogenase (G6PD) deficiency, rather than mishandling of the transfused RBC unit.
3. **How could the event have been prevented in this particular case?** Appropriate discussion regarding the antibody findings in this patient with the clinical team prior to transfusion could have prevented the nonimmune hemolysis of the RBC unit that occurred. The patient's physician could have been advised that the cold-reacting autoanti-I antibody will not cause in vivo hemolysis since it is reactive only at temperatures well below 37 °C, thus avoiding the need to warm the RBC unit prior to transfusion. Furthermore, the clinical team, including the nurse, should be instructed in acceptable and safe methods for warming blood components such as allowing the unit to sit at room temperature for 30–40 min and/or the use of an approved blood warmer which is set and alarmed to maintain the temperature within a safe range of 37–42 °C.

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Chapter 42

Baby Fate



Clinical History

A 24-year-old woman, gravida 1, para 0 (G1P0), at 38 weeks of gestation presents to labor and delivery for spontaneous rupture of membranes. Her pregnancy was uneventful. A baby girl is delivered 13 h later by normal spontaneous vaginal delivery. The baby's Apgar scores are 8 and 9 at 1 and 5 min, respectively. Shortly after being taken to the nursery, the nurse notices that the baby has diffuse petechiae on the face, scalp, and shoulders. The mother's hemoglobin is 12.8 g/dL, and the platelet count is 182 K/ μ L. Meanwhile, the neonatal hemoglobin is 19.8 g/dL, and the platelet count is 32 K/ μ L. Samples for both the mother and the baby (cord blood sample) (ethylenediaminetetraacetic acid [EDTA] anticoagulant) are submitted to the blood bank.

ABO/Rh/Antibody Screen

Patient

ABO/Rh (gel method)			Patient plasma (reverse typing)	
Patient RBCs (forward typing)				
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	4+	0	4+
Antibody screen (AHG/gel method)				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Neonate (Cord Blood)

ABO/Rh (tube method)			
Cord RBCs			
Anti-A	Anti-B	Anti-D	Weak D
0	0	4+	NT
Cord anti-IgG DAT: 0			
Reaction scale = 0 (no reaction) to 4+ (strong reaction)			

Questions

1. What is the apparent cause of the baby’s thrombocytopenia?
2. What is the differential diagnosis for a newborn with thrombocytopenia?
3. What options are there for treating the baby?
4. Is neonatal thrombocytopenia likely to recur in future pregnancies from this mother? If so, what testing should be performed?

Answers

1. **What is the apparent cause of the baby’s thrombocytopenia?** The baby has a very low platelet count while the mother’s platelet count is normal. This is a typical scenario for fetal and neonatal alloimmune thrombocytopenia (FNAIT). FNAIT is an uncommon disease, but is the leading cause of severe thrombocytopenia in the newborn. FNAIT can have significant sequelae, including intracranial hemorrhage (ICH), although most cases are mild [1, 2]. Unlike hemolytic disease of the fetus and newborn (HDFN), FNAIT can occur in the first pregnancy and is often not discovered until delivery. Platelet antigens are expressed early in gestation; thus, the mother is exposed to paternal platelet antigens on the platelets of the developing fetus through a mechanism that is not yet understood. The mother can then develop antiplatelet antibodies which will cross the placenta and destroy the fetal platelets.
2. **What is the differential diagnosis for a newborn with thrombocytopenia?** Autoimmune thrombocytopenia (i.e., idiopathic [immune] thrombocytopenic purpura [ITP], usually passively acquired from the mother), drug-related destruction of platelets, infection, necrotizing enterocolitis, disseminated intravascular coagulation (DIC), hypersplenism, Kasabach–Merritt syndrome (giant hemangioma with thrombocytopenia and consumptive coagulopathy), thrombosis, and rare genetic abnormalities are all possible causes of neonatal thrombocytopenia.

3. **What options are there for treating the baby?** Severe thrombocytopenia (platelets less than 30 K/ μ L) or signs of bleeding in the first 24 h of life must be addressed because of the risk of neurological sequelae from ICH. Platelet counts should be maintained at greater than 100 K/ μ L for the first 72–96 h of life. For neonates known to be affected, when the antibody is known (or anti-HPA-1a is highly suspected; note that anti-HPA-1a is the culprit antibody in the majority of cases—see table below [3]), antigen-negative platelets can be obtained from the blood supplier. Alternatively, maternal platelets can be collected for the anticipated delivery; the maternal platelets must be washed and irradiated prior to transfusing the baby. This is effective because the mother's platelets are negative for the offending antigen (i.e., HPA-1a antigen negative) and washing will remove the antibodies. However, timing of the platelet collection for the delivery can be challenging, and collecting platelets from a perinatal woman is not ideal. If it was not known that the infant was affected, as in this case, and/or HPA-1a-antigen-negative platelets are not available, then “off-the-shelf” platelets can be used. FNAIT-affected neonates can be safely and successfully treated with regular donor platelets even though they are likely to be antigen positive. Intravenous immunoglobulin (IVIG) can be administered as adjunctive therapy as it can reduce the time it takes for the baby's platelet count to recover.

Prevalence of maternal antibodies in FNAIT	
Antibody	Frequency (%)
HPA-1a	79
HPA-5b	9
HPA-1b	4
HPA-3a	2
HPA-5a	1

4. **Is neonatal thrombocytopenia likely to recur in future pregnancies from this mother? If so, what testing should be performed?** Yes, the recurrence rate for FNAIT is very high (90%). Thus, in order to have appropriate platelets available or to perform intrauterine platelet transfusion in severe cases, it is beneficial to determine which platelet antigen is the culprit. The most common antibody implicated is anti-HPA-1a, which is found in 75–80% of cases (see table above). Although between 1.6% and 4.6% of the population is negative for HPA-1a antigen, only 10% of these patients will develop an antibody. To screen for FNAIT, flow cytometry is first used to detect the presence of immunoglobulin (Ig)G and IgM platelet-reactive antibodies. For identification of the specific antibody, a solid-phase assay, such as modified antigen-capture enzyme-linked immunosorbent assay (MACE) or monoclonal antibody immobilization of platelet antigens (MAIPA), is used.

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Chapter 43

Thromboelastography, My Dear Doctor



Clinical History

A 63-year-old man with a history of myocardial infarction and unstable angina is scheduled for coronary artery bypass graft (CABG) surgery with cardiopulmonary bypass (CPB). A preoperative ethylenediaminetetraacetic acid (EDTA) anticoagulant sample is submitted to the blood bank for type and screen along with a request for crossmatch of four units of red blood cells (RBCs) in preparation for the surgery.

ABO/Rh/Antibody Screen

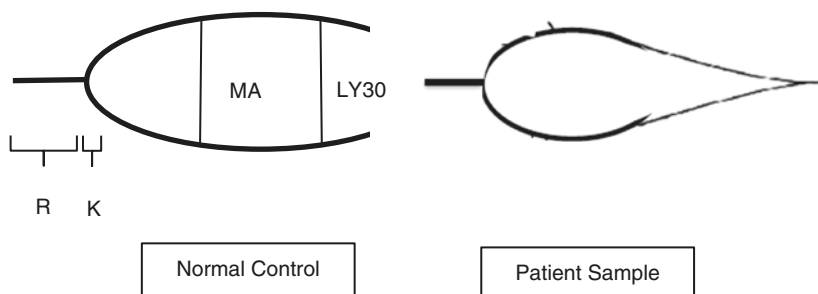
<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	4+	3+	0	0
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4 + (strong reaction)

Additional History

The CABG surgery is completed without complication, but immediately postoperatively, the patient is noted to have persistent oozing from the surgical site despite administration of protamine sulfate to reverse heparin anticoagulation. The

activated clotting time (ACT) is 100 s (normal range, 90–150 s). The anesthesiologist places a stat order for two units of apheresis platelets believing that the cause of the ongoing bleeding is platelet dysfunction, a common issue in CPB. In the meantime, the patient's sample is sent for thromboelastography (also known as viscoelastic testing); 35 min later, the following graph is produced:



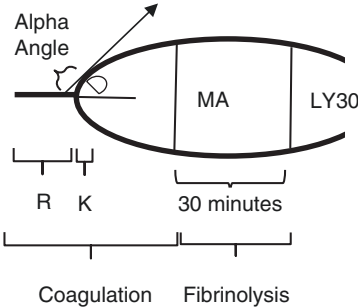
Questions

1. What is the ACT and how does it differ from the activated partial thromboplastin time (aPTT)?
2. What is the principle of thromboelastography?
3. Based on the patient's thromboelastograph shown, compared to the normal control graph, is the anesthesiologist correct in requesting apheresis platelets to stop the patient's persistent oozing from the surgical site? If not, what blood products or therapeutic agents might be effective?

Answers

1. **What is the ACT and how does it differ from the aPTT?** The ACT is a point-of-care test that is similar to the aPTT test but is used to monitor high-dose unfractionated heparin (UFH) anticoagulation typically used for CPB or extracorporeal membrane oxygenation (ECMO). Unlike the aPTT, the ACT can measure clot formation using a small volume of fresh whole blood from the patient (0.5–1 mL vs. 4.5 mL of citrated plasma for the aPTT). Similar to the aPTT, the ACT uses a negatively charged activator (such as celite, kaolin, or glass beads) to initiate clotting through activation of factor (F)XII, though the normal range of the ACT is longer than for the aPTT (typically 90–150 s vs. 32–40 s, respectively). The ACT can be prolonged by issues other than UFH, including severe thrombocytopenia, lupus anticoagulant, and factor deficiency (especially deficiency of contact factors: FXII, prekallikrein, and high-molecular-weight kininogen).


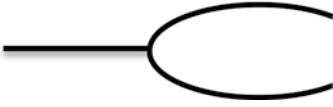


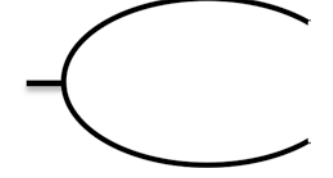


2. **What is the principle of thromboelastography?** Thromboelastography (commonly referred to as TEG® [1]) is a point-of-care test which analyzes the viscoelastic properties of blood during clot formation under low-shear stress. A fresh citrated blood sample is placed into a cup (or cuvette), and as the cup rotates (or oscillates), a suspended pin measures clot parameters, including time to clot initiation, time to clot formation, clot strength, and clot retraction or fibrinolysis. Thus, all stages of clotting, including the interaction of platelets, are measured by thromboelastography. Thromboelastometry (commonly referred to as rotational thromboelastometry or ROTEM® [2]) is similar to thromboelastography, except that the pin rotates rather than the cup. The diagram below shows a typical thromboelastograph and its hemostatic measurements.



Parameter		Normal range
Reaction time (R)	Time to clot initiation	2–8 min
Kinetic time (K)	Clot formation	1–3 min
Maximum amplitude (MA)	Maximum amplitude (clot strength/firmness)	55°–78°
Lysis (LY30)	Clot lysis at 30 min after MA	51–69 mm
Alpha (α) angle	Slope between R and K (rate of clot formation)	0–8%

3. **Based on the patient’s thromboelastograph shown, compared to the normal control graph, is the anesthesiologist correct in requesting apheresis platelets to stop the patient’s persistent oozing from the surgical site? If not, what blood products or therapeutic agents might be effective?** The patient’s thromboelastograph shows a pattern of hyperfibrinolysis (some diagnostic thromboelastograph patterns are shown in the table below); thus, platelet transfusion would not be the correct choice for treatment of this patient’s persistent bleeding. An antifibrinolytic agent (i.e., intravenous [IV] tranexamic acid [10 mg/kg] or IV epsilon aminocaproic acid [5 g]) would be a better choice for treatment of this patient [3, 4]. Also, if necessary, cryoprecipitate can be used to replace fibrinogen (note that there is a human plasma-derived fibrinogen concentrate available in the United States, but it is approved by the Food and Drug Administration

[FDA] only for treatment of acute bleeding in patients with congenital fibrinogen deficiency, though there is interest in off-label use for hemorrhagic emergencies [5, 6]).

Thromboelastography pattern	Interpretation/causes	Parameters
	Normal	$R, K, MA,$ $\alpha = \text{Normal}$
	Anticoagulants, hemophilia/ factor deficiency	$R, K = \text{Prolonged}$ $MA, \alpha = \text{Decreased}$
	Thrombocytopenia, thrombocytopathy (platelet inhibitor medications)	$R = \text{Normal}$ $K = \text{Prolonged}$ $MA = \text{Decreased}$
	Hyperfibrinolysis, tissue plasminogen activator (tPA)	$R = \text{Normal}$ $MA = \text{Decreased}$ $LY30 = \text{Increased}$
	Hypercoagulation	$R, K = \text{Decreased}$ $MA = \text{Increased}$
	Disseminated intravascular coagulation (DIC) (hypercoagulable state with secondary fibrinolysis)	$R, K = \text{Decreased}$
	DIC (hypocoagulable state)	$R, K = \text{Prolonged}$ $MA = \text{Decreased}$

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Chapter 44

Spoiled Rotten!



Clinical History

A 34-year-old woman with a history of idiopathic (immune) thrombocytopenic purpura (ITP) presents to the emergency department (ED) with persistent epistaxis and purpura on her upper and lower extremities. The patient has received intravenous immunoglobulin (IVIG) therapy in the past, but she reports that she has not been compliant with her follow-up visits to her hematologist. The platelet count in the ED is 9 K/ μ L. A type and screen (ethylenediaminetetraacetic acid [EDTA] anticoagulant) sample is submitted to the blood bank along with a request for 12 units of platelets.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

The blood bank technologist is concerned about the order for 12 units of platelets since the blood bank maintains only two units of single donor (apheresis) platelets in their inventory for the 250-bed community hospital; the ED physician becomes upset when told that only two platelet units are available. The laboratory manager subsequently contacts the ED physician to clarify the request after which, the blood bank technologist is instructed to issue the two units of single donor platelets to the ED for transfusion to the patient. Twenty minutes into the transfusion of the first unit of platelets, after approximately half of the product has been transfused, the patient develops fever (temperature increase from 98.6 to 101.8 °F) and shaking chills. The platelet unit is returned to the blood bank along with a posttransfusion (EDTA anticoagulant) blood sample from the patient; a transfusion reaction workup is performed.

Clerical check			
Patient: O-positive		Donor unit: O-positive	
Visual check: no hemolysis			
	Polyspecific	Anti-IgG	Anti-C ₃ d
DAT (pre-sample)	0	NT	NT
DAT (post-sample)	0	NT	NT

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Further History

The patient is admitted to the hospital. Intravenous (IV) antibiotics are initiated, and the second unit of single donor platelets is successfully transfused with a posttransfusion platelet count of 27 K/ μ L. Blood cultures taken in the ED from the patient at the time of the transfusion reaction grow out *Staphylococcus epidermidis* which matches cultures obtained from the residual platelet unit associated with the transfusion reaction.

Questions

1. Why was the blood bank technologist concerned about the platelet order, and what was likely discussed between the laboratory manager and the ED physician to clarify the request?
2. What is the apparent cause of the transfusion reaction in this case, and why are platelet products more susceptible to this type of reaction? Is there any role for cold storage (i.e., storage at 1–6 °C) of platelets?
3. What measures have been taken to prevent this type of reaction?

4. What reporting responsibility does the transfusion service medical director have in this case? What is the reporting responsibility of the blood donor collection facility that provided the platelet component?

Answers

1. **Why was the blood bank technologist concerned about the platelet order, and what was likely discussed between the laboratory manager and the ED physician to clarify the request?** A fairly common miscommunication occurs between clinicians and blood bank staff in the ordering of platelet transfusions. This is because many clinicians tend to order platelet units in terms of platelet concentrates derived from whole blood (often referred to as “random donor platelets”), while many blood banks maintain inventories stocked with apheresis platelets (commonly referred to as “single donor platelets”). A single donor platelet unit, in fact, contains about six times the number of platelets contained in a random donor platelet unit (3.0×10^{11} vs. 5.5×10^{10} platelets per unit, respectively). Thus, random donor platelet units are typically pooled together (on average, six units are pooled into one bag) in order to transfuse a therapeutic dose equivalent to a single donor unit. As a result, the blood bank technologist was rightfully concerned that the ED physician ordering the platelets did not understand the difference between random donor and single donor platelets. Fortunately, the laboratory manager was able to explain the difference between the two types of platelet products and that transfusion of two single donor platelet units is equivalent to 12 random donor units. If in fact one did actually attempt to transfuse 12 units of single donor platelets, there would be a high risk of volume overload given that the patient would receive nearly 2.5 L of fluid (or about half the whole blood volume of an average-sized adult).
2. **What is the apparent cause of the transfusion reaction in this case, and why are platelet products more susceptible to this type of reaction? Is there any role for cold storage (i.e., storage at 1–6 °C) of platelets?** It is evident from the cultures of the patient’s blood sample and the residual platelet product that the patient suffered from a reaction caused by bacterial contamination of the apheresis platelet unit. Platelets are more susceptible to bacterial contamination because of room temperature (RT, 20–24 °C) storage (platelet concentrates may be stored at RT for up to 5 days with continuous gentle agitation). Although many different species of bacteria, gram-positive and gram-negative, aerobic and anaerobic, can be involved in contamination of platelet concentrates, gram-positive aerobes such as *Staphylococcus* species (especially those that are coagulase negative) tend to be the most common isolates involved, though cases involving gram-negative bacteria are more frequently fatal [1]. Meanwhile, cold storage of platelets complies with the US Food and Drug Administration (FDA) Code of Federal Regulations (CFR), Title 21 Sections 640.24 and 640.25, which allows for storage of whole blood platelet concentrates at 1–6 °C with or without

gentle agitation [2, 3]. Cold storage (CS) reduces the risk of bacterial contamination. In fact, transfusion of CS platelets was the standard of care in the United States from the 1960s through the mid-1980s [2]. However, since it was recognized that RT-stored platelets have a longer in vivo survival time, they became the standard of care and have been particularly favored for the treatment of hypoproliferative thrombocytopenia (i.e., prophylactic treatment in nonbleeding patients). Currently, there is renewed interest in CS platelets since cold storage preserves the immediate hemostatic effect of transfused platelets necessary for the treatment of acute hemorrhages (i.e., CS platelets are “primed” for rapid hemostasis, whereas RT platelets exhibit increased aggregation defects requiring 1–2 h posttransfusion circulation for recovery of hemostasis) [2]. CS platelets may also have the advantage of a longer shelf life (up to 10 days, though this has not been approved by the FDA) [2]. Maintaining inventories of both RT and CS platelets would be ideal; however, most hospital blood banks would have significant difficulty facing the challenge of maintaining a dual platelet inventory.

3. **What measures have been taken to prevent this type of reaction?** Traditionally, aside from proper aseptic donor phlebotomy technique and handling of platelet units, the main measure taken by the FDA to prevent reactions and fatalities caused by bacterial contamination (i.e., septic transfusion reactions) was to reduce the allowed storage time of platelet concentrates from 7 to 5 days (this change occurred in the mid-1980s) [4]. Platelet swirling was another popular and rapid method used in the past by transfusion services to detect bacterial contamination [5]. Swirling involves rotating the platelet unit in front of a light source; platelets that have not been activated retain the discoid shape and will give off a wave or swirl while activated platelets (i.e., platelets that have changed shape owing to low pH or temperature) will not swirl. Naturally, swirling is quite subjective. Thus, estimated rates of contaminated platelet units remained quite high (1 in 2000–3000 units) with septic reactions estimated to occur in 1 in 25,000 platelet transfusions [6]. To address the problem, the AABB (formerly, the American Association of Blood Banks) adopted a standard in 2004 requiring measures to limit and detect bacterial contamination in all platelet components [7]. This prompted donor collection centers to initiate routine quality-control testing for the detection of bacteria in apheresis platelet concentrates at the time of collection which reduced but did not entirely eliminate the risk of septic reactions [6]. Additional measures later taken included improved donor arm disinfection through the use of chlorhexidine-based cleansing solution in place of iodine-based preparations and the use of diversion-pouch technology to prevent the skin plug from entering into the primary collection bag (instead, the skin plug [which harbors many bacteria] is diverted into a small side pouch during collection of the first few milliliters of blood flow) [7]. There is still ongoing discussion about the role of point-of-release screening for bacterial contamination with some centers utilizing this secondary testing to extend the shelf life of their apheresis platelet inventory to 7 days (which has been approved by the FDA) [8, 9]. Pathogen inactivation (PI) technology, which had been used in Europe for quite some time prior to receiving approval from the FDA for use in the United

States, is yet another option to achieve improved safety of platelet transfusions [10]. PI involves photochemical treatment of platelet concentrates via the addition of photoactive substances, such as amotosalen (INTERCEPT® Blood System; Cerus Corporation, Concord, CA) and riboflavin (Mirasol® Pathogen Reduction Technology; Terumo BCT, Lakewood, CO), followed by exposure to ultraviolet light (UVA or UVB, depending on the system used) [10]. PI technology improves the safety profile of platelet concentrates through inactivation of all kinds of pathogens, including bacteria and viruses (especially enveloped viruses) as well as protozoan parasites (including *Babesia microti* and *Trypanosoma cruzi*); moreover, since PI technology can prevent white blood cell (WBC) activation, it is effective in the prevention of transfusion-associated graft-versus-host disease (TA-GVHD) [11]. However, although PI technology appears to be safe and cost-effective [10], the clinical effectiveness of PI-treated platelets is still under debate as there are concerns surrounding increased platelet activation and reduced survival of PI platelets [11].

4. **What reporting responsibility does the transfusion service medical director have in this case? What is the reporting responsibility of the blood donor collection facility that provided the platelet component?** Reporting of the case to regulatory authorities is dependent on the outcome of the patient as well as the locality in which the transfusion service is located. The transfusion service medical director must report the event to the blood collection facility which provided the component; the donor collection facility, in turn, must submit a Biological Product Deviation Report (BPDR) to the FDA/Center for Biologics Evaluation and Research (CBER) within 45 days of notification (this is typically done via the online reporting system) [12]. In the event of a fatal outcome, the medical director would also be obligated to report the case to the FDA immediately by telephone with follow-up of a written fatality report within 7 days detailing the case. Notification of state and city health departments may also be required depending on the location of the transfusion service. Finally, the transfusion service may voluntarily participate in and report to the National Healthcare Safety Network (NHSN) Hemovigilance Module (see Chap. 24, question 4 answer for more information on reporting, including the NHSN Hemovigilance Module).

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Chapter 45

Bad Blood



Clinical History

A 48-year-old man with a history of pancreatic cancer who underwent distal pancreatectomy and splenectomy 4 months ago presents with fatigue, weakness, pallor, and dyspnea on exertion. On hospital admission, the patient is found to be severely anemic with a hemoglobin (Hgb) of 5.6 g/dL and a lactate dehydrogenase (LDH) level of 1180 U/L, total bilirubin level of 6.4 mg/dL, haptoglobin level less than 7 mg/dL, and a reticulocyte count of 16.2%. The blood bank history shows that the patient had received two units of red blood cells (RBCs) post pancreatectomy (one on postoperative day #1 and the second on postoperative day #2); the preoperative antibody screen was negative. A type and screen (ethylenediaminetetraacetic acid [EDTA] anticoagulant) sample is submitted to the blood bank along with a request for crossmatch of two units of RBCs for transfusion.

ABO/Rh/Antibody Screen

ABO/Rh (gel method)				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	4+	0	4+	0
Antibody screen (AHG/Gel method)				
SC1	3+			
SC2	3+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel Panel

Rh-hr		Kell							Duffy		Kidd	Lewis		MNS			P	Lutheran	Test results												
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG Gel		
1	R ₁ R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	+	0	+	+	+	+	+	0	+	3+	
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	+	3+	
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	0	+	+	0	0	0	+	0	+	+	+	0	+	+	3+
4	R ₀ r	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	0	0	0	+	0	0	+	+	+	+	+	0	+	+	3+
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	+	0	0	+	0	+	+	0	+	+	3+
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	+	+	+	+	0	+	+	3+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	0	+	+	+	0	0	+	0	+	+	+	0	+	+	3+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	0	+	+	0	+	+	3+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	0	+	+	0	0	+	0	+	0	0	0	+	+	+	3+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	0	+	+	3+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	0	+	+	0	0	+	+	0	0	+	+	0	+	+	3+
Patient cell																															3+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

DAT profile			
Polyspecific	3+	Anti-IgG	3+
Reaction scale = 0 (no reaction) to 4+ (strong reaction)			
		Anti-C ₃ d	0

Acid Eluate Panel

		Rh-hr										Kell				Duffy		Kidd	Lewis		MNS				P	Lutheran	Test results				
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P _I	Lu ^a	Lu ^b	AHG	CC	
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	3+	NT
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	0	+	+	+	+	+	0	0	+	3+	NT
3	R ₂ R ₂	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+	+ ^S	0	+	3+	NT
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	0	0	0	+	+	+	+	+	0	+	3+	NT
5	r' r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	+	0	+	3+	NT
6	r'' r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	+	+ ^w	0	+	3+	NT	
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+ ^S	0	+	3+	NT	
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	+	+ ^S	0	+	3+	NT	
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	+	+	0	0	+	0	0	0	0	+	3+	NT
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	+	+ ^w	0	+	3+	NT	
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	+	3+	NT
Last wash SC1																											0		2+		
Last wash SC2																											0		2+		

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

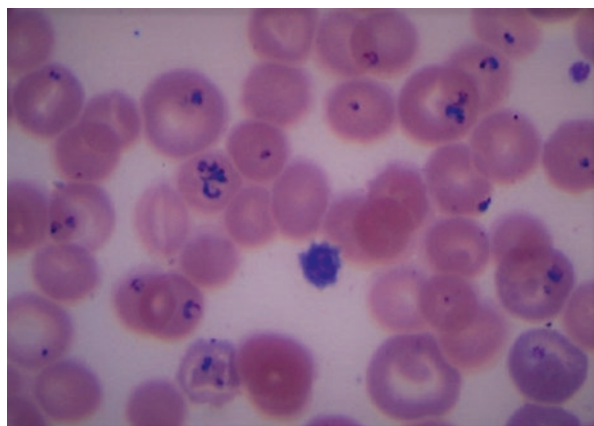
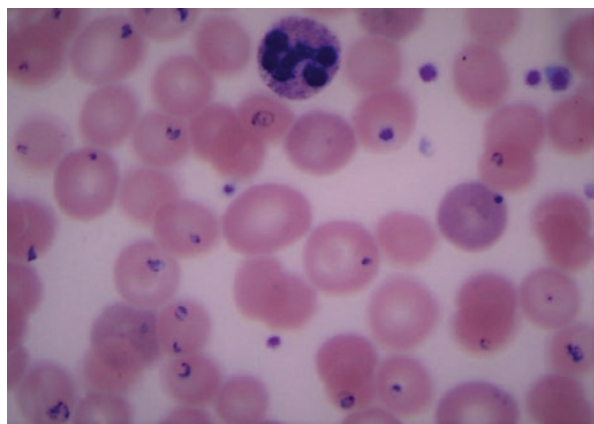
Autoadsorption Panel

		Rh-hr							Kell				Duffy		Kidd	Lewis		MNS			P	Lutheran		Test results						
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P _I	Lu ^a	Lu ^b	AHG	CC
1	R ₁ W R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	0	+	2+
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	0	0	+	+	+	0	0	+	0	2+
3	R ₂ R ₂	+	0	+	0	0	0	0	0	0	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	+	2+
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	0	0	0	0	+	+	+	+	+	0	+	2+
5	r' r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	0	+	2+
6	r'' r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	0	0	+	+	0	+	+	+	+	+	+	0	+	2+
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	0	+	2+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	+	+	+	+	0	+	+	+	+	+	0	+	2+
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	0	0	0	0	+	0	+	0	0	+	0	+	2+
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	+	+	+	0	+	2+
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	+	2+
Patient cell																												0		2+

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Additional History

The patient is transfused one “least incompatible” RBC unit with a subsequent rise in the Hgb to 6.2 g/dL. Treatment with prednisone 1 mg/kg/day is started for “Coombs-positive” hemolytic anemia. The following day, a peripheral blood smear is reviewed as below:



Peripheral blood thin smear: 100× Oil, Wright-Giemsa stain. Photomicrograph credit: Malarly Mani, MD, Department of Pathology, Mount Sinai St. Luke’s-West, New York, NY

Further History

Based on the peripheral blood smear findings, further history is obtained. On detailed questioning of the patient, he is noted to be a city dweller who has not traveled to any suburban or rural area within the prior 8 months, and he denies any insect (i.e., tick or mosquito) bites or unusual rashes. The patient denies ever receiving a transfusion of blood or blood products prior to the pancreatic surgery.

Questions

1. What is the significant finding on the peripheral blood smear? In consideration of this finding and in light of the patient's clinical history, what is the most probable source of transmission to the patient? What reporting responsibility does the medical director of the transfusion service have in this case?
2. What is the relation of the patient's immunohematology results to the peripheral blood smear finding?
3. How does the patient's history of receiving a splenectomy as part of his pancreatic cancer surgery affect his current condition?
4. Should treatment with therapeutic apheresis be considered for this patient? If so, what type of therapeutic apheresis procedure should be performed?
5. What measures have been taken by blood donor centers to prevent what occurred in this case?

Answers

1. **What is the significant finding on the peripheral blood smear? In consideration of this finding and in light of the patient's clinical history, what is the most probable source of transmission to the patient? What reporting responsibility does the medical director of the transfusion service have in this case?** The peripheral blood smear shows intraerythrocytic, ring-form, protozoan parasites consistent with babesiosis (*Babesia microti*). Given that the patient does not have a significant travel risk or known tick bite, the most probable source of the parasitic infection is one of the postoperative blood transfusions that the patient received (babesiosis is typically transmitted through the bite of the deer tick, *Ixodes scapularis*, but can be transmitted via blood transfusion or by vertical transmission as well; babesiosis is endemic in parts of the United States [of which *B. microti* species is most common], particularly in the northeast and upper Midwest regions [1]. Based on this, the medical director of the transfusion service must report such transmission to the blood collection facility for further investigation of the involved blood donors. In addition, there may be reporting requirements to the regional and/or local (i.e., state and/or city) departments of health; babesiosis has been classified by the Centers for Disease Control and Prevention (CDC) as a nationally notifiable disease since 2011 [2]. On a technical note, it may be quite difficult to differentiate babesiosis from malaria (in particular, *Plasmodium falciparum*) on peripheral blood smear alone, though some features that favor the former are greater variation in ring forms (note the variation in the smear above), lack of hemozoin pigment, absence of schizonts and gametocytes, presence of extraerythrocytic parasites, and the finding of the classic Maltese cross form (which is not often found) [3]. In addition, in the United States, where this case occurred, babesiosis is much more common than malaria. It should also be noted that both thick and thin smears should be examined in actual practice [3].

2. **What is the relation of the patient's immunohematology results to the peripheral blood smear finding?** The patient in this case presents with warm autoimmune hemolytic anemia (WAIHA) as evidenced by the clinical, laboratory, and immunohematology findings, including the positive anti-IgG direct antiglobulin test (DAT) result. WAIHA has been described in association with cases of babesiosis [4, 5].
3. **How does the patient's history of receiving a splenectomy as part of his pancreatic cancer surgery affect his current condition?** Asplenic patients are at increased risk of having more severe infection with babesiosis, as are older and immunocompromised patients (e.g. human immunodeficiency virus [HIV] infection) [5].
4. **Should treatment with therapeutic apheresis be considered for this patient? If so, what type of therapeutic apheresis?** Yes, RBC exchange transfusion may be considered for a patient with babesiosis infection in conjunction with antimicrobial therapy, particularly when the patient has very high parasitemia (greater than 10%) or when the patient manifests other comorbidities of infection such as hemolysis, disseminated intravascular coagulation (DIC), or pulmonary or renal or hepatic compromise [6]. RBC exchange transfusion is currently classified as a category II level indication (disorders for which apheresis is accepted as second-line therapy, either as a standalone treatment or in conjunction with other modes of treatment) for babesiosis by the American Society for Apheresis (ASFA) [6]. Since this particular patient is at higher risk of more severe infection, as a result of the splenectomy, and depending on the parasite load (which appears to be quite high on the peripheral blood smear), RBC exchange transfusion might be a consideration for the patient in this case.
5. **What measures have been taken by blood donor centers to prevent what occurred in this case?** Although traditionally prospective blood donors have been screened for babesiosis only via the donor health questionnaire, some blood donor centers, particularly those in endemic regions of the United States, more recently have initiated donor testing under investigational protocol; note that the United States Food and Drug Administration [FDA] has approved tests to screen for *B. microti* in human plasma and whole blood samples in March 2018 [2, 7]. These tests include both antibody (IgG arrayed fluorescence immunoassay [AFIA]) and polymerase chain reaction (PCR) nucleic acid test [NAT]) assay-based testing. Donors who test positive for babesiosis or have a history of babesiosis (even if treated) are permanently deferred from donating blood for another person.

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Chapter 46

Emergency!



Clinical History

A 22-year-old man is brought to the emergency department after sustaining a motor vehicle accident and hemorrhagic shock. The massive transfusion protocol (MTP) is activated, and the patient receives two trauma packs (each pack containing five units of uncrossmatched group O-negative red blood cells [RBCs], five units of type AB plasma, and one unit of apheresis platelets [any ABO type]). Subsequently, a type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank.

ABO/Rh/Antibody Screen

ABO/Rh (gel method)				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
mf	0	0	0	4+
Antibody screen (AHG/Gel method)				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Questions

1. What is the patient’s ABO/Rh blood type?
2. What is the principle of the MTP?

3. Supposing that the patient has stabilized but needs additional RBC transfusion subsequent to receipt of the type and screen sample, should the patient be given cross-match type-specific blood or continue to be given group O RBCs? Regarding the patient's Rh(D) type, should the patient be given Rh-positive or Rh-negative blood?
4. In consideration that frozen plasma can take 30 min or longer to thaw in preparation for transfusion, causing undue delay in the handling of emergencies, how should the plasma inventory for MTPs be managed?

Answers

1. **What is the patient's ABO/Rh blood type?** The patient is very likely to be group A-negative though the front-typing results show a mixed field effect due to the quantity of group O blood transfused during resuscitation and the MTP.
2. **What is the principle of the MTP?** MTPs are employed to rapidly replace large quantities of lost blood. Massive transfusion can loosely be defined as replacement of total whole blood volume in 24 h, transfusion of 10 or more RBC units in 24 h, or blood loss at a rate of greater than 150 mL/min. There are several approaches to MTPs. In the laboratory-driven approach, blood products (especially, platelets and plasma) are reserved until the laboratory values (e.g., platelet count, prothrombin time [PT], activated partial thromboplastin time [aPTT]) become significantly abnormal to warrant their transfusion. In the protocol-driven approach (used in this case), products are transfused according to a set ratio, usually a 1:1:1 ratio of RBCs to plasma to platelets (i.e., five units of RBCs, five units of plasma, and one unit of apheresis platelets [equivalent to five units of whole blood-derived platelets, often referred to as "random donor platelets"]). The latter approach came into favor after some retrospective data touted benefit in war casualties [1]. Subsequently, a prospective randomized control trial showed that there was no difference in overall mortality between trauma patients receiving massive transfusion in a 1:1:1 ratio compared to 1:1:2 ratio [2]. However, there were fewer hemorrhagic deaths in the 1:1:1 group. Massive transfusion can exacerbate a number of complications in trauma, such as hypothermia, dilutional thrombocytopenia, coagulopathy, and metabolic imbalances (hypocalcemia, hyperkalemia, and acid-base disturbances).
3. **Supposing that the patient has stabilized but needs additional RBC transfusion subsequent to receipt of the type and screen sample, should the patient be given crossmatch type-specific blood or continue to be given group O RBCs? Regarding the patient's Rh(D) type, should the patient be given Rh-positive or Rh-negative blood?** Although there may be some reluctance to switch the patient from group O blood because of the mixed-field results on front typing, one may crossmatch group A RBCs since anti-A is absent in the back type. This would help to conserve group O blood. Regarding Rh(D)-positive versus Rh(D)-negative blood, although the patient is Rh-negative, there may be issues regarding inventory levels of Rh-negative blood, particularly if the patient

is experiencing ongoing bleeding and requiring further support with RBC transfusion. In the case of male or elderly patients who are Rh-negative and not sensitized (i.e., do not have anti-D antibodies), it is acceptable to use Rh-positive RBCs. On the other hand, in the case of an Rh-negative female of childbearing age, it is preferable to use Rh-negative RBCs to the extent possible (clearly, one must use Rh-positive blood in the event that there is ongoing life-threatening bleeding and the Rh-negative inventory has been depleted).

4. **In consideration that frozen plasma can take 30 min or longer to thaw in preparation for transfusion, causing undue delay in the handling of emergencies, how should the plasma inventory for MTPs be managed?** It is worthwhile to briefly consider plasma ABO type for support of MTPs; since frozen plasma requires at least 30 min to thaw in a 37 °C water bath, trauma centers must often keep thawed plasma available in the refrigerator at 1–6 °C in order to have plasma immediately ready for trauma use. However, it is clearly impractical to maintain inventories of thawed plasma for each ABO blood type or to maintain high inventories of thawed type AB plasma (since AB plasma is typically in short supply), and so, some trauma centers have adopted the practice of initially using group A plasma until the patient's blood type has been determined. There are some reports that such use of emergency-release pre-thawed type A plasma does not significantly affect clinical outcomes despite transfusion of incompatible plasma while such a strategy conserves type AB plasma [3, 4].

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Chapter 47

Time to Change the Plasma



Clinical History

A 54-year-old woman presents to the emergency department because of altered mental status. She has a history of hypertension and breast cancer and weighs 65 kg. The patient has no prior history of transfusion. Her laboratory values are significant for a hematocrit (Hct) level of 20%, platelets 12 K/ μ L, lactate dehydrogenase (LDH) level 2500 U/L, and creatinine 2.3 mg/dL. The prothrombin time (PT) and activated partial thromboplastin time (aPTT) laboratory values are within the normal range. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank along with a request for two units of red blood cells (RBCs). In addition, urgent therapeutic plasma exchange (TPE) is requested.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	4+	0	0	0
<i>Antibody screen (AHG/Gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Questions

1. What is the patient's ABO/Rh blood type?
2. What is the most likely cause of the patient's clinical condition? What additional laboratory testing would you request to confirm the diagnosis?
3. Based on the clinical presentation, what would you expect to find on the peripheral blood smear?
4. How many units of plasma will be needed in order to accomplish a single plasma volume exchange for this patient (each unit of plasma is approximately 300 mL in volume)?
5. In consideration of the patient's blood type, if there is a shortage of compatible plasma for this patient, what options are there for TPE and treatment of the patient's condition?

Answers

1. **What is the patient's ABO/Rh blood type?** The patient is group AB-negative. Refer to Chap. 1, question 1 for further information on forward and reverse ABO typing.
2. **What is the most likely cause of the patient's clinical condition? What additional laboratory testing would you request to confirm the diagnosis?** Based on the clinical presentation (altered mental status) and the laboratory findings of hemolytic anemia with thrombocytopenia and renal insufficiency, the patient likely has thrombotic thrombocytopenic purpura (TTP). TTP is characterized by microangiopathic hemolytic anemia and thrombocytopenia caused by deficiency of a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) metalloprotease (an enzyme that cleaves von Willebrand factor [VWF] multimers). Therefore, initial laboratory testing should include an ADAMTS13 level which is typically less than 10% in TTP; often, an inhibitor to ADAMTS13 is the cause of the deficiency. In this setting, when renal failure is present, if the ADAMTS13 level is greater than 10%, one may suspect an alternative diagnosis of atypical hemolytic uremic syndrome (aHUS). The cause of aHUS is related to inhibitors to or genetic mutations of complement factors (e.g., complement factor H or I). Eculizumab is a humanized monoclonal antibody that binds to C5 complement and acts as a terminal complement inhibitor; it is indicated for treatment of aHUS as well as paroxysmal nocturnal hemoglobinuria and generalized myasthenia gravis [1, 2]. Refer to Chap. 35, question 1 answer for more information on the topic of TTP and aHUS.
3. **Based on the clinical presentation, what would you expect to find on the peripheral blood smear?** Schistocytes are characteristically found, along with thrombocytopenia, though they are not pathognomonic for TTP as schistocytes can be present in other conditions such as disseminated intravascular coagulation (DIC) or even postoperatively.

4. **How many units of plasma will be needed in order to accomplish a single plasma volume exchange for this patient (each unit of plasma is approximately 300 mL in volume)?** To calculate this patient's plasma volume, first multiply 65 kg (patient's weight) by 70 mL/kg (estimated whole blood volume per kg in an adult) to get the total blood volume of 4550 mL. Next, since the patient's Hct is 20%, multiply 4550 mL by $1 - 0.20$ or 4550 multiplied by 0.80 to get the plasma volume of 3640 mL.

Then divide 3640 by 300 (approximate volume of a unit of plasma) to get the estimated number of plasma units needed for a single plasma volume exchange (this is often helpful when initiating TPE in order to prepare the plasma in advance of the treatment). In this case, 12 units are necessary.

5. **In consideration of the patient's blood type, if there is a shortage of compatible plasma for this patient, what options are there for TPE and treatment of the patient's condition?** Unfortunately, type AB plasma units may often be in short supply given that only 1–2% of the population has AB blood type. Therefore, finding compatible plasma for this patient who requires daily TPEs can be quite challenging. However, several strategies may be used to manage this patient's plasma exchanges. First, one should consider using different types of plasma products that may be available such as cryodepleted plasma (essentially, plasma supernatant after cryoprecipitate has been removed) and solvent–detergent plasma (a plasma product that is chemically treated to inactivate lipid-enveloped viral pathogens such as human immunodeficiency virus [HIV], hepatitis B virus [HBV], and hepatitis C virus [HCV]; nonenveloped viruses such as parvovirus B19 are not inactivated [also see Chap. 40 question 4 answer]). A second possibility is to consider using type A plasma as part of the exchange volume (often, the anti-B titer is low enough in type A plasma so as not to cause hemolysis in the patient). Yet a third consideration is to use albumin solution or combination of albumin with normal saline in the first part of the exchange, finishing the exchange with plasma. This strategy allows for removal of the ADAMTS13 inhibitors in the first part of the exchange while replacing ADAMTS13 enzyme in the latter part. Finally, aggressive treatment with steroids or other immunosuppressive agent (such as rituximab [monoclonal anti-CD20/B-cell marker antibody]) may also benefit in reducing the number of TPE's necessary for recovery.

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Chapter 48

Do the Math!



Clinical History

A 72-year-old man is admitted to the hospital with a presumptive diagnosis of acute myeloid leukemia. He is scheduled for bone marrow biopsy but has a platelet count of 7 K/ μ L (7000/ μ L). A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank along with a request for two units of apheresis platelets.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/Gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

The patient is transfused with two units of A-positive irradiated apheresis platelets in the morning. An evening platelet count result, however, is 12 K/ μ L (12,000/ μ L). The following morning, the platelet count is 8 K/ μ L (8000/ μ L) and a unit of O-positive irradiated apheresis platelets is transfused; approximately 1 h after transfusion, the platelet count is repeated and found to be 14 K/ μ L (14,000/ μ L).

Because of the poor response to platelet transfusion, human leukocyte antigen (HLA)-matched platelets are requested for the patient by the hematologist.

Questions

1. What are the causes of refractoriness to platelet transfusion?
2. Considering the patient's ABO type, what effect do the ABO types of the transfused platelet units have on the posttransfusion response?
3. If the patient weighs 50 kg and is 5 feet 8 inches tall (172 cm), calculate the 1-h posttransfusion corrected count increment (CCI) based on the pre- and posttransfusion platelet counts using the formulas below (use 3.0×10^{11} as the number of platelets in an apheresis platelet unit). Based on your calculation, would you recommend HLA-matched platelets for this patient?

$$\text{Body surface area (BSA)} (\text{m}^2) = \sqrt{(\text{height} \times \text{weight}) / 3600}$$

$$\text{CCI} = \frac{(\text{posttransfusion} - \text{pretransfusion platelet count } [/\mu\text{L}]) \times \text{BSA}}{\text{number of transfused platelets } (\times 10^{11})}$$

4. What additional laboratory testing is necessary and/or recommended before provision of HLA-matched platelets?
5. Supposing that the patient is found to have antibodies to human platelet antigens (HPA, e.g., anti-HPA-1a) in addition to HLA antibodies, what other options are there for platelet transfusion?

Answers

1. **What are the causes of refractoriness to platelet transfusion?** There are immune and nonimmune causes for non-responsiveness (i.e., refractoriness) to platelet transfusion. Nonimmune causes, which are much more common, include underlying conditions such as fever, medications, splenomegaly, disseminated intravascular coagulation (DIC), and sepsis. Under such circumstances, the underlying condition must be corrected or stopped in order for the platelet count to rise appropriately after transfusion (generally, 30–50 K/ μL). Immune refractoriness, on the other hand, occurs because the patient has developed antibodies that rapidly destroy the transfused platelets. Most commonly, the antibodies are anti-HLA (platelets express class I HLA) though they may also be anti-HPA. A 1-h posttransfusion platelet count is a sensitive screening method due to this rapid clearance of the transfused platelets. The management of patients who have immune refractoriness to platelets is quite challenging as these patients are

often bleeding or at risk of bleeding, but do not respond to transfusion with standard platelet products. Using ABO-compatible platelets is a first step; platelets have A and B antigens on their surface and so may have shortened posttransfusion survival if there is ABO incompatibility with recipient A and B isoantibodies (see question 2 answer below). Subsequently, HLA-matched or crossmatched platelets (see below) may be required to manage the patient. It should be noted that one major study comparing transfusion of pooled, random donor platelets versus apheresis (single donor) platelets and the development of immune refractoriness were equivalent as long as the patient had been receiving leukocyte-reduced blood products (platelets and RBCs) [1]. Leukocyte reduction prevents HLA alloimmunization.

2. **Considering the patient's ABO type, what effect do the ABO types of the transfused platelet units have on the posttransfusion response?** It is important to remember that when transfusing platelets, ABO type does have significance. First, because platelets are suspended in donor plasma, there is the possibility of recipient hemolysis in the case of a minor incompatibility. For example, this would occur in transfusion of group O platelets to a group A patient (i.e., donor plasma anti-A reacting with recipient RBC A antigen, see Chap. 16, question 1 answer). On the other hand, major incompatibility, and potentially shortened survival of platelets, may occur in the case of group A platelets transfused to a group O recipient; platelets express A, B, and H antigens. Thus, in this case, the survival of the first two units of platelets, both group A, transfused to the group O patient may have been affected by the patient's anti-A.
3. **If the patient weighs 50 kg and is 5 feet 8 inches tall (172 cm), calculate the 1-h posttransfusion CCI based on the pre- and posttransfusion platelet counts using the formulas below (use 3.0×10^{11} as the number of platelets in an apheresis platelet unit). Based on your calculation, would you recommend HLA-matched platelets for this patient?**

$$\text{Body surface area (BSA)} (\text{m}^2) = \sqrt{(\text{height} \times \text{weight}) / 3600}$$

$$\text{CCI} = \frac{(\text{posttransfusion} - \text{pretransfusion platelet count} [\mu\text{L}]) \times \text{BSA}}{\text{number of transfused platelets} (\times 10^{11})}$$

Plugging in the numbers into the above formulas,

$$\text{BSA} = 1.55 \text{ m}^2; \text{CCI} = [(14,000 / \mu\text{L} - 8000 / \mu\text{L}) \times 1.55] / 3.0 (\times 10^{11}) = 3100.$$

In general, a 1-h CCI ≤ 7500 is indicative of immune refractoriness. Thus, HLA-matched platelets may be necessary for this patient, though most recommendations state that the CCI should be tested on at least two occasions.

4. **What additional laboratory testing is necessary and/or recommended before provision of HLA-matched platelets?** In order for HLA-matched platelets to

be ordered for the patient, the patient's HLA type (class I, HLA-A and HLA-B antigens) need to be known. Thus, if not previously done, the patient's sample needs to be sent for HLA typing. In addition, it is recommended to test the patient's sample for the presence of HLA and HPA antibodies. If such testing is negative, HLA-matched or crossmatched platelets may not be effective in increasing the platelet count. HLA antibody testing may be accomplished through cell-based (such as complement-dependent cytotoxicity) and solid phase (such as enzyme-linked immunosorbent assay [ELISA] or flow cytometry) methods, the latter having increased sensitivity over the former [2]. Even so, the success of HLA-matched platelets will also depend on the level of the match; that is to say that A-level matches (perfect match for all four HLA-A and HLA-B antigens) and BU-level matches (no HLA-A or HLA-B antigen differences because of homozygosity) will fare better than BX-level (one antigen mismatch within same cross-reactive group) or C-level (one non-cross-reactive antigen mismatch) matches [3].

5. **Supposing that the patient is found to have antibodies to HPA (e.g. anti-HPA-1a) in addition to HLA antibodies, what other options are there for platelet transfusion?** Under such circumstances, when both HLA and HPA antibodies are present, or in case that the patient exhibits a high HLA-panel-reactive antibody (HLA-PRA; i.e., the patient has multiple HLA antibodies), reacting patient's serum against donor platelets may be useful in finding platelets that are most compatible with the patient's antibodies. Methods for platelet crossmatching include solid-phase red-cell adherence (SPRCA), modified antigen capture ELISA (MACE), and flow cytometry [2]. In other instances, use of crossmatched platelets may be valuable as an interim measure while awaiting the results of HLA antigen typing of the patient, which otherwise would cause delay in transfusing compatible platelets. Yet another strategy for managing highly refractory patients involves antigen avoidance (i.e., transfusion of platelets lacking HLA antigens to which the recipient is known to have antibodies against) [3].

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Chapter 49

Eight is Enough!



Clinical History

A 34-year-old man with hemophilia A is scheduled for tooth extraction. His hematologist recommends increasing the factor (F)VIII (FVIII:C) level to 50% immediately prior to the extraction. The patient weighs 66 kg, and the patient's FVIII level is currently 5%.

Questions

1. What is the dose of recombinant FVIII that the patient requires to achieve the desired level?
2. Suppose that the patient has hemophilia B, what is the dose of recombinant FIX that the patient requires to achieve a FIX level of 50% if the current level is 5%?

Answers

1. **What is the dose of recombinant FVIII that the patient requires to achieve the desired level?** The formula to calculate the dose of recombinant FVIII concentrate is:

$$\text{Dosage required (IU)} = \text{Body weight (Kg)} \times \frac{\text{Desired FVIII increase (IU/dL or \% normal)}}{0.5} \times \text{0.5 (IU/kg per IU/dL)}$$

Plugging in the numbers from the case:

$$\begin{aligned}\text{Dose required (IU)} &= 66 \text{ kg} \times (50\% - 5\%) \times 0.5 \text{ IU / kg per IU / dL} \\ &= 66 \times 45 \times 0.5 = 1485 \text{ IU.}\end{aligned}$$

However, it is important to closely monitor FVIII levels since pharmacokinetics and clinical response may vary [1].

2. **Suppose that the patient has hemophilia B, what is the dose of recombinant FIX that the patient requires to achieve a FIX level of 50% if the current level is 5%? The formula to calculate the dose of recombinant FIX concentrate is:**

$$\begin{array}{ccccccc}\text{Dosage} & & & & \text{Desired} & & \\ \text{required (IU)} & = & \text{Body weight} & \times & \text{FIX increase} & \times & \text{Reciprocal of observed} \\ & & \text{(Kg)} & & \text{(IU/dL or \%} & & \text{recovery} \\ & & & & \text{normal)} & & \text{(IU/kg per IU/dL)}\end{array}$$

Once again, plugging in the numbers from the case and using 1.3 IU/kg per IU/dL as the reciprocal (since average recovery in adults is 0.8 IU/kg):

$$\begin{aligned}\text{Dose required (IU)} &= 66 \text{ kg} \times (50\% - 5\%) \times 1.3 \text{ IU / kg per IU / dL} \\ &= 66 \times 45 \times 1.3 = 3861 \text{ IU.}\end{aligned}$$

Note that in pediatric patients (less than 15 years), the average recovery is 0.7 IU/kg, so the reciprocal to use is 1.4 rather than 1.3 IU/kg per IU/dL. However, in all cases, it is important to closely monitor FIX levels since pharmacokinetics and clinical response may vary [2].

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Chapter 50

It's So "Vonderful"



Clinical History

A 51-year-old woman with breast cancer and a history of type 1 von Willebrand disease (VWD) is scheduled for a left total mastectomy. The patient relates a history of easy bruising, frequent nose bleeds, and menorrhagia; the patient successfully delivered a full-term baby at age 32 for which she had received prophylactic transfusions with cryoprecipitate. The patient has had no prior surgeries, but did have a wisdom tooth extraction as a teenager for which she received prophylactic desmopressin acetate (DDAVP, 1-deamino-8-D-arginine vasopressin) injection and experienced only minor bleeding. A type and screen (ethylenediaminetetraacetic acid [EDTA] anticoagulant) sample is submitted to the blood bank.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction); W weak

Additional History

Preoperative workup results show that the patient's von Willebrand factor/ristocetin cofactor (VWF:RCo) activity level is 30 IU/dL (normal range 50–200 IU/dL) and the factor (F)VIII (FVIII:C) level is 65% (normal range 50–150%). The prothrombin time (PT), activated partial thromboplastin time (aPTT), and the platelet count are all within the normal range. Based on the patient's history, laboratory results, and weight (the patient weighs 72 kg), the patient is given an injection of DDAVP (0.3 µg/kg) 30 min prior to the surgery, and the mastectomy is performed without any complications. Postoperatively, the patient receives the first dose of human plasma-derived VWF concentrate (2800 IU) 2 h after the surgery followed by two subsequent 1400 IU doses at 8 h apart. On postoperative day #1, the VWF concentrate frequency is tapered to every 12 h. Repeat testing shows that the VWF level is 80 IU/dL while the FVIII level is 155%.

Questions

1. What is the role of VWF in coagulation? What is VWD?
2. What is the relation between the patient's ABO group and VWF level?
3. What is the mechanism of DDAVP in the treatment of VWD? Is DDAVP effective for most types of VWD patients?
4. Why has the patient's FVIII level risen to such a high level (155%)? Is there any risk to the patient because of this high FVIII level? What precaution could have been taken to prevent the level of FVIII from reaching such a high level?

Answers

1. **What is the role of VWF in coagulation? What is VWD?** VWF serves two important functions in coagulation: (1) VWF is essential for platelet adhesion and (2) VWF is a specific carrier of FVIII in plasma [1]. VWF is often referred to as ristocetin cofactor (ristocetin is an antibiotic that was discontinued for clinical use once it was discovered that it caused VWF-induced platelet aggregation [2]). VWD is the most common inherited bleeding disorder (incidence of 66–100 cases per million in the general population) [2]. However, VWD is not a uniform disorder as there are six subtypes that are currently classified plus a platelet type (see Table below) [1, 3, 4]. In addition to inherited VWD, a rare acquired form also exists [5].

VWD Type	Inheritance	Description
Type 1	Autosomal dominant	All VWF multimers present but in lower amounts (partial quantitative deficiency)
Type 2A	Autosomal dominant	Absence of high-molecular-weight VWF multimers

VWD Type	Inheritance	Description
Type 2B	Autosomal dominant	Absence of high-molecular-weight VWF multimers but increased affinity of VWF to platelet glycoprotein (GP)Ib α
Type 2M	Autosomal dominant	Qualitative abnormality: variants with decreased platelet-dependent function and normal multimeric structure
Type 2N	Autosomal recessive	Qualitative abnormality: abnormal binding of FVIII to VWF; may be difficult to distinguish from mild hemophilia A
Type 3	Autosomal recessive	Severe deficiency of VWF
Platelet-type	Autosomal dominant	Mutation in gene encoding for platelet GPIb α ; similar to VWD type 2B

- What is the relation between the patient's ABO group and the VWF level?**
The patient's blood type is group O; group O individuals have VWF levels that are 25% lower than non-group-O individuals [6].
- What is the mechanism of DDAVP in the treatment of VWD? Is DDAVP effective for most types of VWD patients? DDAVP is a synthetic analog of arginine vasopressin that acts by causing release of VWF from endothelial storage sites known as Weibel–Palade bodies (note that VWF is also stored in platelet alpha granules) into the circulation. However, DDAVP is typically only effective as an initial therapy since repeated dosing will not be effective once VWF has been depleted from the endothelial storage sites (i.e., tachyphylaxis). Additionally, while DDAVP is not effective for all types of VWD, it is typically effective for treatment of mild type 1 VWD, the most common type, accounting for greater than 50% of cases by some sources [1].**
- Why has the patient's FVIII level risen to such a high level (155%)? Is there any risk to the patient because of this high FVIII level? What precaution could have been taken to prevent the level of FVIII from reaching such a high level?** It is apparent that the main reason for the patient's supra-elevated FVIII:C level is related to the treatment with the human plasma-derived VWF concentrate. This is because such concentrate also contains significant amounts of FVIII, and, in fact, depending on the specific manufacturer product, the product may also be indicated for the treatment of hemophilia A [7, 8]. However, it is worth recalling that FVIII levels also tend to rise in the presence of inflammatory states (i.e., FVIII is an acute phase reactant), which may contribute to the supra-elevated levels. Supra-elevated FVIII:C levels have been associated with venous thrombosis, so such levels are not without risk to the patient [9]. Besides careful monitoring of VWF and FVIII:C levels in conjunction with replacement therapy, recombinant VWF concentrate (which has been recently approved by the Food and Drug Administration [FDA] for use in the United States) could be useful as such concentrate contains no FVIII [10].

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Chapter 51

Cruising for a Bruising



Clinical History

A 41-year-old woman with a history of menorrhagia is referred to the emergency department (ED) because of severe anemia, hemoglobin (Hgb) level 5.5 g/dL, and dyspnea on exertion. The patient has had multiple pregnancies but no transfusions and reports taking no medications other than over-the-counter multivitamins. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank along with a request for transfusion of two units of red blood cells (RBCs).

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

The patient is transfused with two units of immediate-spin (IS)-compatible O-positive RBCs and discharged home from the ED. Two days later, the patient returns to the ED with a complaint of nose bleeding and upper and lower extremity patchy red-purple skin discolorations. The patient is afebrile and the Hgb level has improved to 7.0 g/dL, but the platelet count is markedly low (16 K/ μ L; previously 175 K/ μ L); the prothrombin time (PT) and activated partial thromboplastin time (aPTT) are normal (12.0 s and 33.0 s, respectively). The direct antiglobulin test (DAT) is negative and the lactate dehydrogenase (LDH) level is normal (310 U/L); a peripheral blood smear confirms marked thrombocytopenia with normal erythrocyte morphology.

Questions

1. What causes of thrombocytopenia should be considered in this patient, and what is the most likely cause?
2. How would you support your diagnosis of the most likely cause of thrombocytopenia in the patient? How does the severe thrombocytopenia occur in this condition?
3. How would you treat this patient's thrombocytopenia? Is platelet transfusion an option?
4. What does the term "hemovigilance" mean and how does it relate to this case?
5. Suppose this is a patient who is considering pregnancy sometime in the future, what risk should be discussed with this patient in light of the patient's current situation?

Answers

1. **What causes of thrombocytopenia should be considered in this patient, and what is the most likely cause?** There are many etiologies of thrombocytopenia to consider, including medications such as heparin, idiopathic (immune) thrombocytopenic purpura (ITP), fever, sepsis, splenomegaly, disseminated intravascular coagulation (DIC), and thrombotic thrombocytopenic purpura (TTP). However, all of these can be excluded based on the history (the patient is not taking any medications and is afebrile) while laboratory values do not support a diagnosis of DIC (normal PT and aPTT values) or TTP (there is no evidence of hemolytic anemia based on the normal LDH value and no finding of schistocytes on the peripheral smear). Meanwhile, ITP would also be unlikely given the rapid decline in platelets after transfusion. Therefore, based on the clinical setting,

posttransfusion purpura (PTP) is the most likely cause of the severe thrombocytopenia. Of note, PTP usually occurs 5–9 days after transfusion, though it may occur sooner (as in this case). It is also of interest that this case occurred after RBC transfusion; it is important to note that cellular products such as RBCs contain small amounts of platelets which can trigger PTP [1].

How would you support your diagnosis of the most likely cause of thrombocytopenia in the patient? How does the severe thrombocytopenia occur in this condition? In order to definitively diagnose PTP, one must find antibodies to specific human platelet antigen (HPA) and lack of the corresponding antigen on the patient's platelets. Most commonly, anti-HPA-1a is the cause in a patient whose platelets lack the very common HPA-1a antigen (the incidence of the HPA-1a-negative phenotype in Caucasians is about 2.5%) [2]. The antibodies typically develop as a result of exposure during pregnancy (and, thus, the condition is more common in females) or occasionally secondary to transfusion. However, it is unknown why severe thrombocytopenia occurs after exposure to transfused antigen-positive platelets since the recipient's own platelets are antigen-negative, but are also being destroyed in the process. Accordingly, three theories have been postulated to account for this autologous platelet destruction: (1) destruction through immune complex formation, (2) destruction through soluble antigens, and (3) destruction through antibody autoreactivity.

2. **How would you treat this patient's thrombocytopenia? Is platelet transfusion an option?** Intravenous immunoglobulin (IVIG) remains the current treatment of choice though steroids and therapeutic plasma exchange (TPE) are also options in severe cases (the American Society for Apheresis [ASFA] lists TPE as a category III indication for PTP [optimum role of apheresis not established], though the level of evidence for this recommendation is weak, grade 2C) [3]. Platelet transfusion should be avoided (unless there is severe hemorrhage) until recovery, which usually occurs within 4 days after IVIG. Fortunately, PTP does not typically recur with subsequent transfusions after recovery.
3. **What does the term "hemovigilance" mean and how does it relate to this case?** The term "hemovigilance" refers to a set of surveillance procedures that are used to report and monitor transfusion-related events in order to improve transfusion safety. Hemovigilance was started in France in 1994 and spread to the United Kingdom (where it is termed "Serious Hazards of Transfusion" or "SHOT") and eventually to the United States in 2010 through The National Healthcare Safety Network (NHSN) Hemovigilance Module [4, 5]. Unlike France, however, reporting to the NHSN Hemovigilance Module is strictly voluntary which limits its overall effectiveness. The NHSN Hemovigilance Module includes a case definition for PTP, and of note in this case, the imputability (which is the likelihood of causation of the event by the transfusion) would be assigned as "probable" given that the event occurred less than 5 days after transfusion. Furthermore, data reported to the UK's SHOT system suggested that the universal leukoreduction may reduce the incidence of PTP.
4. **Suppose this is a patient who is considering pregnancy sometime in the future, what risk should be discussed with this patient in light of the patient's**

current situation? Given that the patient most probably has anti-HPA-1a as the cause of PTP, there is a risk during pregnancy that the baby will be affected by the antibody and be thrombocytopenic. This is known as fetal and neonatal allo-immune thrombocytopenia (FNAIT) and may result in neonatal intracranial hemorrhage and death [6]. See Chap. 42 for more information on FNAIT.

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Chapter 52

Help, I Cannot Stop the Bleeding!



Clinical History

A 78-year-old man is brought to the emergency department (ED) after falling on ice and sustaining head trauma outside of his home. A computed tomography (CT) head scan shows intracranial hemorrhage (ICH). The patient is noted to have a medical history significant for nonvalvular atrial fibrillation for which he takes rivaroxaban anticoagulant medication 20 mg orally once daily. The patient is reported to weigh 65 kg. Coagulation laboratory studies show prothrombin time (PT) 17.2 s and activated partial thromboplastin time (aPTT) 30.0 s. A type and screen (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank along with a stat request for four units of fresh frozen plasma (FFP).

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Questions

1. In consideration of the clinical scenario and the order for FFP, what are your recommendations regarding management of this patient's coagulation status?
2. Suppose that instead of rivaroxaban, the patient was taking daily warfarin anti-coagulant medication and that the patient's PT is 24.4 s in the ED. The testing laboratory uses a thromboplastin reagent with an assigned international sensitivity index (ISI) of 1.2 to determine the PT values, and the geometric mean of the normal PT range is 13.0 s. What dose of nonactivated four-factor prothrombin complex concentrate (PCC, Kcentra® [CSL Behring, LLC, Kankakee, IL]) should be administered along with intravenous vitamin K (10 mg) in order to reverse the warfarin anticoagulation? How would the recommended Kcentra® dose change if the patient weighed 108 kg?

Answers

1. **In consideration of the clinical scenario and the order for FFP, what are your recommendations regarding management of this patient's coagulation status?** This clinical scenario is becoming more common as novel anticoagulants (NOACS), such as dabigatran etexilate (an oral direct thrombin inhibitor [DTI]) and rivaroxaban, apixaban, and edoxaban (oral direct factor Xa inhibitors), replace the use of oral vitamin K antagonists (warfarin) in the United States. Rivaroxaban may prolong the PT (as in this patient) and/or the aPTT, but use of such laboratory monitoring is not reliable nor recommended (though specialized laboratory testing, such as a chromogenic anti-Xa assay, may prove to be useful for select high-risk patients) [1, 2]. While NOACS have more predictable pharmacology such that routine monitoring is unnecessary (unlike warfarin which requires routine monitoring of the INR) and do not have interaction with various foods, the Xa inhibitors unfortunately do not have an available antidote in the event of significant hemorrhage. Warfarin, on the other hand, may be reversed with vitamin K and four-factor PCC administration while the monoclonal antibody, idarucizumab, has been approved for reversal of dabigatran [3]. The anticoagulant effect of rivaroxaban is not generally reversed with transfusion of FFP; therefore, the blood product order in this case is inappropriate. There is limited evidence that activated charcoal may be of benefit if given within 1–6 h of the drug ingestion [1, 3]. Meanwhile, since much of the drug remains bound to plasma proteins after ingestion, hemodialysis cannot remove the drug. Finally, administration of activated PCC or nonactivated PCC (at a dose of 50 IU/kg not to exceed the maximum dose of 5000 IU) may partially reverse the effect of rivaroxaban anticoagulation which has a half-life of approximately 10–12 h, depending on the age, health, and renal and hepatic functions of the person taking the drug [1, 3, 4]. Other Xa inhibitors (apixaban and edoxaban) have similar

pharmacodynamics [5, 6]. Clearly, this recommendation will change in the event that a reversal agent is approved for Xa inhibitors similar to the one approved for dabigatran (as of this writing, antidotes, such as andexanet alfa and ciraparantag [which may also reverse heparin], are in development) [3, 7].

2. **Suppose that instead of rivaroxaban, the patient was taking daily warfarin anticoagulant medication and that the patient's PT is 24.4 s in the ED. The testing laboratory uses a thromboplastin reagent with an assigned international sensitivity index (ISI) of 1.2 to determine PT values, and the geometric mean (mean) of the normal PT range is 13.0 s. What dose of nonactivated four-factor PCC (Kcentra®) should be administered along with intravenous vitamin K (10 mg) in order to reverse the warfarin anticoagulation? How would the recommended Kcentra® dose change if the patient weighed 108 kg?** Nonactivated four-factor PCC is indicated for reversal of warfarin anticoagulation in patients who are experiencing life-threatening bleeding or prior to urgent/emergent surgery in conjunction with vitamin K administration [8]. Therefore, it is clearly indicated for this patient who has been found to have ICH on the head CT scan. The appropriate dose of four-factor PCC is dependent on the degree of the international normalized ratio (INR) elevation and the patient's weight: 25 IU/kg (maximum 2500 IU) for an INR between 2.0 and 4.0, 35 IU/kg (maximum 3500 IU) for an INR between 4.0 and 6.0, and 50 IU/kg (maximum 5000 IU) for an INR exceeding 6.0. However, the INR is not given in this case (admittedly, this is rather unusual since coagulation analyzers automatically calculate the INR) but must be calculated using the patient's PT value (24.4 s), the geometric mean normal range of PT (13.0 s), and the thromboplastin ISI (1.2, this is the degree of purity of the reagent compared to the standard thromboplastin assigned an ISI of 1.0). Using the formula:

$$\text{INR} = (\text{Patient's PT} / \text{mean normal range PT})^{\text{ISI}}$$

$$\text{INR} = (24.4 \text{ s} / 13.0 \text{ s})^{1.2} = 2.1288 \text{ (or 2.13 rounded off)}$$

Based on this INR, a dose of 25 IU/kg should be administered for a total of 25 IU/kg \times 65 kg = 1625 IU. Note that the PCC international units (IU) refers to the factor (F)IX content in the product; four-factor PCC contains FII (prothrombin), FVII, FIX, and FX along with protein C, protein S, and antithrombin III. This differs from three-factor PCC/FIX complex concentrates (Bebulin® [Baxalta US Inc., Westlake Village, CA], Profilnine® SD [Grifols Biologicals Inc., Los Angeles, CA]), which lack significant amounts of FVII and activated PCC (anti-inhibitor coagulant complex) which contains activated FVII (FVIIa; FEIBA® [Baxter Healthcare Corporation, Westlake Village, CA]). Kcentra® PCC also contains heparin; thus, it is specifically contraindicated for patients who have heparin-induced thrombocytopenia [7]. If the patient weighed 108 kg instead of 65 kg, the Kcentra® dose would be calculated as 25 IU/kg \times 108 kg = 2700 IU. However, since this exceeds the maximum recommended dose for the calculated level of INR elevation, only 2500 IU should be administered.

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Chapter 53

Saving Blood



Clinical History

A 67-year-old woman with a history of osteoarthritis is scheduled for elective left hip replacement surgery. She also had hip surgery several years ago at another hospital and was transfused at that time. Her medical history is significant for coronary artery disease, hypercholesterolemia, and hypertension for which she takes statin and antihypertensive medications as well as aspirin and clopidogrel antiplatelet medications (which were discontinued 7 days prior to the surgery). On the day of admission for the hip surgery, the patient is found to have a hemoglobin (Hgb) level of 7.7 g/dL with mean corpuscular volume (MCV) 70 fL, platelets 220 K/ μ L, and prothrombin time (PT) and activated partial thromboplastin time (aPTT) 13.0 s and 39.0 s, respectively. Two units of RBCs are ordered to be transfused preoperatively. The surgeon briefly visits the patient in the preoperative holding area, and consent for the transfusion is obtained as a part of the surgical consent; that is, the patient is asked to initial a box on the consent form to accept all transfusions of blood products related to the surgical procedure. However, transfusion is delayed because of a positive antibody screen and subsequent identification of an anti-K antibody requiring antihuman globulin (AHG) crossmatch. As a result of the delay, only one unit of RBCs is transfused, and the patient is taken into the operating room. The patient loses approximately 1 L of blood during the surgery and is transfused three units intraoperatively. The surgery is otherwise uneventful, and the patient is stable and recovering well postoperatively with Hgb level remaining in the 7–8 g/dL range for 3 consecutive days. However, the patient receives two additional units of RBCs for “acute blood loss anemia” with Hgb level 7.4 g/dL on the day before discharge (a repeat Hgb level is not obtained on day of discharge).

Questions

1. What does the term “patient blood management” (PBM) mean?
2. In this case, what opportunities for PBM may have been missed?

Answers

1. **What does the term PBM mean?** The AABB (formerly, the American Association of Blood Banks) defines PBM as an “evidence-based, multidisciplinary approach to optimizing the care of patients who might need transfusion” [1]. The four guiding principles of effective PBM are anemia management, coagulation optimization, blood conservation, and patient-centered decision-making [2]. Unfortunately, many avoidable transfusions occur despite evidence from randomized, controlled trials demonstrating that restrictive transfusion practices (i.e., maintaining Hgb level at or above 7 g/dL) are as safe as, and perhaps even safer than, liberal transfusion practices (i.e., maintaining Hgb levels at or above 9 or 10 g/dL) in a variety of patient populations, including critical care patients, cardiac surgical patients, orthopedic surgical patients, pediatric intensive care unit (PICU) patients, and patients with gastrointestinal hemorrhage [3–7]. There are a number of reasons for this, including lack of clinical education in transfusion medicine, use of outdated transfusion guidelines, use of outdated transfusion practices (i.e., transfusion of two units of RBCs rather than single-unit transfusion with patient reevaluation), and lack of patient inclusion in transfusion decisions (i.e., not using a shared decision-making approach), among other factors (to be fair, though, not all investigators agree that a restrictive transfusion practice is better than a liberal one in all patient populations, as in one study that concluded that a restrictive transfusion threshold was not superior to a liberal threshold in a study of cardiac surgical patients [8]).
2. **In this case, what opportunities for PBM may have been missed?** PBM includes a number of strategies to minimize the need for transfusions including consent and shared decision-making (i.e., patient discussion regarding the benefits and risks of transfusion as well as alternative treatments), use of updated transfusion guidelines, preoperative anemia management, assessing and optimizing hemostasis (i.e., withdrawing and reversing anticoagulation medications), intraoperative cell salvage and acute normovolemic hemodilution, use of clinical decision support tools such as computer physician order entry (CPOE) to guide transfusion decisions, and provision of transfusion education to clinical staff. In the above case scenario, the patient presented with anemia, possibly iron-deficiency anemia considering the low MCV laboratory value (MCV normal range is 80–100 fL), on the day of surgery; thus, the opportunity to work up and appropriately treat the anemia in advance was missed (ideally, preoperative laboratory testing should occur well in advance of the planned surgery). Instead, blood transfusion was ordered to immediately correct the anemia prior to surgery. Preoperative

anemia is associated with increased risk of blood transfusion; therefore, treatment of anemia well before elective surgery can minimize the need for transfusions. Notably though, the transfusion was delayed due to the patient's positive antibody screen and identification of anti-K on the day of the surgery. This delay can be avoided by sending pretransfusion samples several days prior to the surgery (note that many hospital blood banks will extend the outdate time of the preoperative type and screen sample from 3 days to 7 or more days [in some cases, 21 or even 30 days are accepted] provided that the patient has not received a blood product transfusion or been pregnant within the preceding 3 months).

Meanwhile, consent for the transfusion, taken on the day of surgery, is bundled into the surgical consent and is performed without much apparent discussion leaving little opportunity for the patient to understand the benefits and risks, let alone, the alternatives to transfusion. Clearly, a paternalistic approach (i.e., physician knows best) is taken in this case scenario, contrary to a patient-centered approach in which the patient is involved in the treatment decision. Given that three units of RBCs were transfused intraoperatively in response to the extensive blood loss, cell salvage was apparently not used which could have minimized or avoided the need for the transfusions. Finally, though the patient was in stable condition postoperatively, the "time-honored" two-unit transfusion of RBCs was administered, apparently to increase the Hgb level prior to discharge. Obviously, what is presented here is a "worst-case" clinical scenario; however, it is meant to highlight several practices that should be abandoned in order to implement a successful PBM program [1, 2]. Of note, the AABB and The Joint Commission (TJC, an independent, not-for-profit organization in the United States that accredits healthcare organizations) have teamed up to offer joint certification in PBM to qualified programs (currently offered at three levels of certification, depending on the degree of complexity of the PBM activities offered and the PBM standards that are met) [9].

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Chapter 54

Of Wind and Water



Clinical History

A 65-year-old man with a history of obesity, type 2 diabetes mellitus, chronic anemia, and renal insufficiency is admitted to the hospital for evaluation of worsening renal failure, a drop in the hemoglobin (Hgb) level, dyspnea on exertion, and fatigue. The patient's Hgb is 6.2 g/dL (his baseline is 8.0 g/dL), and the creatinine is 4.4 mg/dL on admission. The patient is tachypneic (respiratory rate is greater than 20 breaths/min) and hypoxic (oxygen saturation is 88% on room air). He is treated with supplemental oxygen 2 L/min by nasal cannula and normal saline by slow intravenous (IV) infusion (125 mL/h). A consult is called for evaluation of gastrointestinal bleeding. In the meantime, a type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank along with a request for two units of red blood cells (RBCs). The patient received RBC transfusion of two units 3 years ago at the hospital; the antibody screen was negative at that time.

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	4+	0	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	1+			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Gel Panel

		Rh-hr										Kell			Duffy			Kidd		Lewis		MNS			P	Lutheran	Test results				
Cell #	Rh-hr	D	C	E	c	e	f	C ^w	V	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	P ₁	Lu ^a	Lu ^b	AHG gel		
1	R _{1w} R ₁	+	+	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	+	0
2	R ₁ R ₁	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	+	+	+	1+
3	R ₂ R ₂	+	+	0	+	0	0	0	0	0	+	0	+	0	+	0	0	+	+	0	0	0	0	+	+	+	+	+	+	+	0
4	R ₀ r	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0	+	+	0	0	+	+	+	+	+	+	+	+	0
5	r'r	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	0	+	+	0	0	0	+	+	+	+	+	+	0
6	r''r	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	0	0	0	+	+	0	0	0	+	+	+	+	+	+	0
7	rr	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	+	+	+	+	+	+	+	+	+	1+
8	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	0	+	+	+	+	+	+	+	+	0
9	rr	0	0	0	+	+	+	0	0	0	+	+	+	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	+	0
10	rr	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	+	+	+	+	+	+	0
11	R ₁ R ₁	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	+	0	+	+	+	+	0
Patient cell																															0

Reaction scale = 0 (no reaction) to 4+ (strong reaction); S strong, W weak

Additional History

Two units of RBCs (A-positive, K-negative) are compatible by antihuman globulin (AHG) crossmatch. The patient receives the first unit of RBCs without any complications. However, after near completion of the second RBC unit (started 30 min after completion of the first unit), the patient begins to cough uncontrollably and gasp for air while his blood pressure increases from 140/90 to 160/100 mmHg. The patient’s temperature remains unchanged at 98.8 °F. The RBC transfusion is immediately discontinued, IV furosemide diuretic (40 mg) is administered, and a sample is submitted to the blood bank for workup of a transfusion reaction. The medicine intern documents the transfusion reaction as “probable TRALI” on the transfusion reaction form submitted along with the posttransfusion sample.

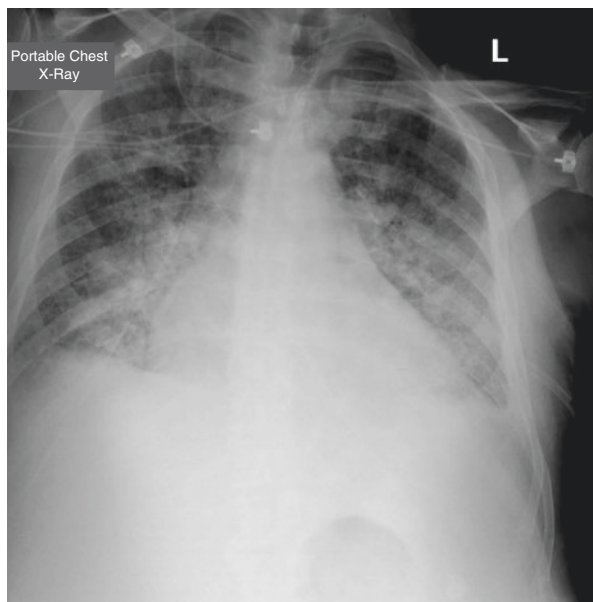
Test Results: Posttransfusion Sample

<i>Clerical check</i>			
Patient: A-positive		Donor units	
		Unit #1	A-positive
		Unit #2	A-positive
<i>Visual check: no hemolysis</i>			
	Polyspecific	Anti-IgG	Anti-C ₃ d
DAT (post-sample)	0	NT	NT

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

The patient is transferred to the medical intensive care unit after being stabilized with a non-rebreather oxygen mask, and a portable chest X-ray is performed 2 h after onset of the acute event. The posttransfusion Hgb is 9.0 g/dL.



Questions

1. Do you agree with the medicine intern's assessment of the transfusion reaction? If not, what is your interpretation of this transfusion reaction?
2. What does the term "TRALI" refer to and how does it occur?
3. What clinical and laboratory information is helpful to determine the underlying cause of the transfusion reaction in this case?
4. Do the patient's antibody panel findings relate in any way to the transfusion reaction event that occurred?
5. How could this transfusion reaction have been prevented?

Answers

1. **Do you agree with the medicine intern's assessment of the transfusion reaction? If not, what is your interpretation of this transfusion reaction?** No, the medicine intern reported the reaction as "probable TRALI" (i.e., transfusion-related acute lung injury); however, the preponderance of available evidence in this case favors volume overload (also referred to as "TACO" or transfusion-associated circulatory overload) though TRALI has not been entirely ruled out. Both feature acute pulmonary edema (which is evident on the portable chest X-ray), but given the amount of fluid volume (both in terms of blood products

and IV normal saline) that the patient was receiving in the setting of worsening renal failure along with the increase in blood pressure, it is more likely that the cause of the pulmonary edema is related to volume overload.

2. **What does the term “TRALI” refer to and how does it occur?** TRALI refers to acute noncardiogenic pulmonary edema, typically occurring within 6 h of transfusion [1]. In simplified terms, the classic mechanism involves donor-related antibodies to human leukocyte antigens (HLA antibodies, class I or II), or less commonly, antibodies to human neutrophil antigens (HNA antibodies) that interact with the corresponding antigens found on the recipient’s leukocytes leading to activation of primed neutrophils with subsequent lung injury and acute bilateral pulmonary edema [2]. This mechanism mostly explains involvement of plasma-containing components (such as fresh frozen plasma) and is the reason behind avoidance of transfusion of plasma at high risk of containing HLA antibodies (i.e., plasma collected from female donors who have been pregnant) [3]. An alternative mechanism involves transfusion of lipids or other biologic response modifiers (BRMs), typically associated with stored cellular products (i.e., platelet concentrates and RBCs), and increased cytokines (interleukins 6 and 8 [IL6 and IL8]) capable of activating primed neutrophils [4, 5]. In some reports, TRALI is explained by a “two-event” model in which priming of the neutrophils occurs secondary to the underlying illness of the recipient while activation occurs secondary to infusion of the leukocyte (HLA or HNA) antibodies or the BRMs [5]. Therefore, because TRALI may occur via an antibody-independent mechanism as well as the fact that presence of HLA antibodies alone may not cause TRALI, it remains a clinical diagnosis that does not absolutely depend on the demonstration of the presence of donor leukocyte antibodies. Aside from acute pulmonary edema that is temporally related to transfusion, TRALI features include absence of evidence of cardiac failure and a ratio of partial pressure of arterial oxygen to the fraction of inspired oxygen ($\text{PaO}_2\text{:FiO}_2$) of less than 300 mmHg with hypoxia, hypotension, fever, and absence of preexisting acute lung injury (ALI) or ALI risk factors (such as sepsis, pneumonia, or aspiration; note that in the presence of preexisting ALI or ALI risk factors, the term “possible TRALI” may be applicable) [5, 6]. Histopathologically, TRALI appears very much like acute respiratory distress syndrome (ARDS) with interstitial and intravascular pulmonary edema, widespread neutrophil infiltration, and hyaline membranes demonstrated in autopsy specimens. However, with aggressive supportive treatment, especially respiratory support, fatalities occur in only a minority of cases (5–10%, though it is a leading cause of transfusion-related fatalities) with resolution generally occurring within 72 h, unlike ARDS [5]. Additionally, occurrence of TRALI does not typically preclude further blood product transfusion since the inciting factors (i.e., HLA antibodies) are related to the specific involved transfused donor product. Steroids are generally not recommended for treatment of TRALI. Finally, the incidence of TRALI is not precisely known but has been variably reported to be as high as 1 per 5000 transfusions to a far lower risk of 1 per 260,000 transfusions for all blood products (with higher risk of 1 per 66,000 for plasma transfusions) [5, 6]. Meanwhile, TRALI risk miti-

gation strategies, including avoidance of transfusion of high-risk plasma and donor HLA-antibody testing, have effectively reduced the incidence [3].

3. **What additional clinical and laboratory information would be helpful to determine the underlying cause of the transfusion reaction in this case?** Additional information that would favor TACO over TRALI include a medical history of congestive heart failure, presence of jugular venous distention (a sign of increased central venous pressure), a positive response to diuretic medication (i.e., increased urinary output), a brain natriuretic peptide (BNP) level greater than 100 pg/mL, and a left ventricular ejection fraction less than 40% on echocardiogram. Meanwhile, no evidence of left atrial hypertension (i.e., circulatory overload), lack of response to diuretic medication, transient neutropenia [7], and the finding of HLA or HNA antibodies in the donor (particularly if the corresponding antigen is detected on the recipient leukocytes) favor a diagnosis of TRALI.
4. **Do the patient's antibody panel findings relate in any way to the patient's transfusion reaction event that occurred?** No, the fact that the patient has developed anti-K as evident in the gel panel does not play any role in the development of either TACO or TRALI.
5. **How could this transfusion reaction have been prevented?** In light of the fact that the most likely cause of the acute pulmonary edema in this case is related to circulatory overload, prevention would have to focus on fluid volume restriction. Thus, minimizing transfusion volume by transfusing only one unit of RBCs instead of two (and adherence to conservative transfusion practices), or even by transfusing a split RBC unit (that is, dividing an RBC unit into two components so that only half the volume is given at one time) is a useful strategy. Slowing the rate of transfusion (i.e., transfusion over 3–4 h) and careful attention to IV fluid administration are other recommended strategies to prevent TACO. Use of diuretic medication may also be of help in prevention of volume overload in susceptible patients. TACO may occur even after transfusion of just a single unit of blood in highly susceptible patients [8].

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Chapter 55

The “Unfraction” Reaction



Clinical History

An 8-year-old boy with congenital heart disease is admitted to the hospital for a planned mitral valve repair and revision. The patient has had multiple admissions to the hospital for surgery and complications relating to the congenital heart condition, most recently 1 month ago, and has required support with multiple transfusions of blood products over the years. His prior blood bank tests show that he is group A-positive with a negative antibody screen. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anticoagulant) is submitted to the blood bank along with a request for crossmatch of two units of red blood cells (RBCs).

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
4+	0	4+	0	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

The child is taken to the operating room and put on cardiac bypass with unfractionated heparin anticoagulation. During the surgery, the patient receives two units of RBCs, two units of fresh frozen plasma (FFP) and a unit of apheresis platelets. Postoperatively, the patient is transferred to the pediatric intensive care unit (PICU). On postoperative day #1, the patient is afebrile with stable vital signs but has a marked drop in his platelet count from 67 K/ μ L to 23 K/ μ L. He is also noted to have discoloration in his left hand with a “dusky” fifth digit; an arterial thrombus in his ulnar artery is identified by ultrasound.

Questions

1. What is the likely cause of the patient’s sudden drop in the platelet count and ulnar arterial thrombosis? Can you explain the pathophysiology behind this complication? Is the timing in this case typical for this complication?
2. Would you expect to see this complication in a child?
3. What are your recommendations for evaluation of this patient?
4. How should this patient be managed?

Answers

- 1. What is the likely cause of the patient’s sudden drop in the platelet count and ulnar arterial thrombosis? Can you explain the pathophysiology behind this complication? Is the timing in this case typical for this complication?**

Heparin-induced thrombocytopenia (HIT) is the likely cause of this patient’s sudden drop in platelet count and ulnar arterial thrombosis. HIT, one of the most clinically important drug-induced complications in hospitalized patients, is a prothrombotic, immunologically mediated complication arising from exposure to unfractionated or even low molecular weight heparin (UFH, LMWH) [1]. With HIT (sometimes referred to as HIT type II to distinguish it from HIT type I which is a nonimmunological form manifested by transient, mild thrombocytopenia occurring within 3 days of heparin exposure), the patient develops moderate thrombocytopenia (or decrease of platelets from baseline [relative thrombocytopenia]) 5–10 days after initial heparin exposure and can develop arterial and venous thrombosis (in fact, 30–60% of adult HIT patients present with thrombotic complications) [2]. HIT results from the formation of antibodies against heparin and platelet factor 4. Platelet factor 4 (PF4) is present in the α -granules of platelets. Following heparin exposure, PF4 binds and neutralizes heparin. This PF4-heparin complex then becomes the “antigen” targeted by an antibody (typically, immunoglobulin [Ig]G class) which recognizes and binds to the exposed epitope, resulting in an immune complex. These complexes bind to

- the FcγIIa receptor on the platelet surface resulting in platelet activation and the formation of platelet microparticles leading to initiation of thrombosis and subsequent thrombocytopenia (or relative thrombocytopenia). The timing of HIT is not typical in this case: although HIT usually occurs 5–10 days post-heparin exposure as noted above, it has been known to occur sooner (within 1–2 days) if the patient has been previously exposed to heparin and has already developed the offending antibodies. In this case, it is plausible that the patient was exposed to heparin during the hospital admission 1 month earlier and developed the antibodies to the PF-4-heparin complex at that time.
2. **Would you expect to see this complication in a child?** HIT occurs much less frequently in the pediatric population but can still cause significant morbidity and mortality. For patients receiving UFH, in adults, the prevalence has been reported to range from 0.5% to 5%, whereas in children, the prevalence of HIT is 0.058%, and HIT with thrombosis is 0.046% [1, 2]. Basically, this means that it may be much less likely to be thought of or recognized in the pediatric population. The 4T scoring system (see table below) has been found to be useful in predicting the pretest probability of HIT as the etiology of thrombocytopenia with or without thrombosis in both populations (though it was not validated for use in pediatric patients, one study has supported its use in this population [1]), with a negative or low-probability score strongly suggestive of another etiology. Use of this scoring system can reduce delays in care and result in reduction of unnecessary testing. Applying the 4T scoring system to this case, the patient would be scored as 2 points for “thrombocytopenia” since there is a 65% fall in platelets (calculated as the difference between the platelet count before and after the fall divided by the starting platelet count multiplied by 100: $[(67 - 23)/67] \times 100 = 65\%$) and the nadir platelets are above 20 K/μL; 2 points for “timing” since the fall in platelets occurred within 1 day of heparin exposure and the patient very likely was exposed to heparin on the prior admission 1 month ago (i.e., within 30 days); 2 points for “thrombosis” since the patient has a new ulnar arterial thrombosis found on ultrasound; and 2 points for “other causes” since there is nothing else in the medical history to explain the sudden drop in platelets experienced by this patient (e.g., the patient does not appear to be septic since he is stable and afebrile). Thus, the total 4T score equals the maximum score of 8, indicative of a high probability for HIT in this case.

4T scoring for heparin-induced thrombocytopenia			
Thrombocytopenia	Platelet count fall ≥50% and platelet nadir ≥20 K/μL	Platelet count fall 30–50% or platelet nadir 10–19 K/μL	Platelet count fall <30% or platelet nadir <10 K/μL
Timing	Clear onset between days 5 and 10 after heparin exposure or within 1 day with prior heparin exposure within 30 days	Consistent with onset days 5–10 after heparin exposure but not clear; platelet count drop after day 10 or platelet count drop ≤1 day with prior heparin exposure 30–100 days ago	Platelet count falls ≤4 days without recent heparin exposure

Thrombosis	New thrombosis, skin necrosis, or acute systemic reaction post-intravenous heparin bolus	Progressive or recurrent thrombosis, non-necrotizing skin lesions, suspected thrombosis (not proven)	None
Other causes for thrombocytopenia	None apparent	Possible	Definite
Points awarded	2	1	0

Probability of HIT	Points
Low probability	≤3
Intermediate probability	4–5
High probability	6–8

3. **What are your recommendations for evaluation of this patient?** Use of the 4T scoring system significantly improves the pretest probability of a true diagnosis of HIT. The diagnosis of HIT is challenging: the main methods of laboratory evaluation for HIT include enzyme-linked immunosorbent assays (ELISA) for the PF4-heparin antibody complex and the functional assay C-serotonin release assay (SRA) [1]. ELISA has a poor specificity (74–86%), and PF4-heparin antibodies can be detected in patients without HIT [1]. The SRA is considered the gold standard as it measures the platelet activation effects of PF4-heparin antibodies with greater than 95% sensitivity and specificity [1]. Unfortunately, the SRA is technically complex to perform and requires special handling of the radioactive ¹⁴C serotonin. The SRA is typically a sendout test only performed in regional labs.
4. **How should this patient be managed?** The most important step in the management of HIT is to immediately discontinue all sources of heparin, including line maintenance (locks, flushes, etc.), and to begin an alternative anticoagulant such as argatroban (an injectable direct thrombin inhibitor). Fortunately, HIT appears to be an acute, self-limited illness with platelet count recovery generally occurring within 1 week of heparin cessation, though the risk of thrombosis continues for several days (and perhaps up to 6 weeks) after discontinuation of heparin [1, 2]. Warfarin should not be used in adults or children with HIT as there is the risk of microthrombosis and warfarin-induced skin necrosis that is caused by protein C depletion occurring out of sync with depletion of other coagulation factors. Platelet transfusions should also be avoided as it may exacerbate the thrombotic effect of HIT.

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Chapter 56

Holy Cow! (“I’m Bloodless”)



Clinical History

A 59-year-old female, a self-described homemaker, and a mother to three children presents to the emergency department (ED) with acute-on-chronic anemia secondary to lower gastrointestinal (GI) bleeding (the patient is known to have a history of colonic diverticular bleeding) and iron deficiency. The patient takes iron and vitamin supplements at home. She additionally takes warfarin due to a history of atrial fibrillation. The patient’s hemoglobin (Hgb) level is 6.4 g/dL in the ED, and the patient appears pale with tachycardia (heart rate 110 beats/min) though her blood pressure is stable at 130/80 mmHg and her oxygen saturation is normal (98%) on room air. A type and screen sample (ethylenediaminetetraacetic acid [EDTA] anti-coagulant) is submitted to the blood bank along with a request for crossmatch of two units of red blood cells (RBCs).

ABO/Rh/Antibody Screen

<i>ABO/Rh (gel method)</i>				
Patient RBCs (forward typing)			Patient plasma (reverse typing)	
Anti-A	Anti-B	Anti-D	A ₁ cells	B cells
0	0	4+	4+	4+
<i>Antibody screen (AHG/gel method)</i>				
SC1	0			
SC2	0			

Reaction scale = 0 (no reaction) to 4+ (strong reaction)

Additional History

A consult is called to further evaluate the GI bleeding. Meanwhile, the ED physician approaches the patient to obtain consent for blood transfusion as per the hospital’s policy. However, the patient’s spouse intervenes and states that his wife has never been transfused and will not accept a blood transfusion citing religious beliefs. The patient nods in agreement, and the spouse further states that he is aware of an “artificial blood substitute” that is available and acceptable to his wife, prompting the ED physician to contact the medical director of the blood bank for advice on how to manage the patient, given that there may be risk of further lower GI bleeding, as well as the fact that the patient’s international normalized ratio (INR) is elevated as a result of warfarin therapy and may need to be corrected using plasma along with vitamin K (the patient’s INR is 2.3 in the ED).

Questions

1. What approach would you recommend to the ED physician regarding the transfusion management of the “bloodless” patient?
2. What obstacles does the patient’s spouse present in regard to the healthcare of the patient?
3. Is the spouse’s assertion that an “artificial blood substitute” is available correct?

Answers

1. **What approach would you recommend to the ED physician regarding the transfusion management of the “bloodless” patient?** It is important for healthcare facilities to have systems in place to identify and manage patients who desire to be treated without the use of blood product transfusions. Patients who conscientiously choose to abstain from receiving blood transfusions, whether on the grounds of religious beliefs (i.e., Jehovah’s Witnesses) or for other personal reasons, can present as some of the most challenging patients to manage. Nevertheless, the first mistake one can make when dealing with such a patient is to take the word “bloodless” at face value or to assume that only RBC transfusions are off limits while non-red cell products are acceptable. It is, therefore, essential to hold a meaningful discussion with the patient regarding their beliefs, wishes, and tolerance limits in regard to blood products. In fact, what one will often find is that such patients have a considerable amount of knowledge concerning blood products and have given much thought to what they will and will not accept under life-threatening circumstances (many will even carry an advance medical directive with them stating what is

acceptable to them, a copy of which should be included in the medical record). While some of these patients may be very stringent, choosing not to accept products derived from blood whatsoever, others may choose to accept blood product derivatives (such as human plasma-derived factor concentrates [e.g., prothrombin complex concentrate (PCC)], cryoprecipitate, or cryosupernatant). For cases in which a surgical procedure is being considered with risk of significant blood loss, the discussion should include intraoperative cell salvage which is accepted by many Jehovah's Witnesses. In the case above, the patient is quite anemic though relatively stable, such that immediate action may not be warranted but that investigation of the possibility of the use of an alternative treatment in case of further bleeding, such as the "artificial blood substitute" referred to by the patient's spouse, may be initiated, especially since it will take some administrative preparation in order to obtain such a product (see discussion below in question 3 answer). Furthermore, consultation by a hematologist could be advised to evaluate the possible roles of intravenous (IV) iron therapy (e.g., iron sucrose or ferric gluconate) and erythropoiesis-stimulating agents in the treatment of this patient. Finally, regarding correction of the INR, withholding of warfarin and administration of IV vitamin K may be adequate measures, though use of four-factor PCC may be discussed with the patient in case it becomes necessary to rapidly reverse the anticoagulant (for discussion about use of four-factor PCC in warfarin reversal, see Chap. 52).

2. **What obstacles does the patient's spouse present in regard to the healthcare of the patient?** Although, the spouse has stated that the patient will not accept a transfusion of blood, what is not clear in this case is whether the patient herself truly holds the same beliefs (note that she is only passively agreeing to the spouse's assertion through a nod of her head). Therefore, one should ask of the patient whether she has a signed advance medical directive; if not, it will be necessary to have a confidential discussion with the patient alone to verify her wishes and to ensure that she understands the possible consequences of her decision to withhold blood products (though one must be respectful of a patient's decision to not accept blood transfusion). Unfortunately, this can be a quite delicate situation since asking the spouse to have a moment alone with the patient may bring on resentment and distrust. Under such circumstances, it may be wise to contact a representative from the Jehovah's Witness Hospital Liaison Committee for assistance.
3. **Is the spouse's assertion that there is an available "artificial blood substitute" correct?** In short, the answer to this question is "yes." However, in reality, obtaining such a product is not quite that easy as there are no readily available products that have been approved by the US Food and Drug Administration (FDA) to date (note that this may imminently change as investigational products are being developed). Thus, products must be obtained under "compassionate use" (also known as "expanded access") circumstances or through an investigational new drug (IND) protocol, if any are ongoing at the time.

Historically, development of oxygen therapeutic agents, which are commonly referred to as "blood substitutes" or "artificial blood," has taken on a two-pronged approach. In one approach, perfluorocarbons (PFCs) are used as the basis for tissue oxygenation. However, although PFCs seemed promising in that they are able to dissolve large amounts of gas (oxygen and carbon dioxide), working with PFCs proved quite difficult since they are hydrophobic and not water-miscible. Thus, they must be emulsified and require ventilation with 100% oxygen (causing concern for toxicity), limiting their clinical effectiveness, and development of second- and third-generation products (such as Oxygent™, originally manufactured by Alliance Pharmaceuticals, San Diego, CA, and Oxycyte™, Tenax Therapeutics, Morrisville, NC) has not come to fruition despite many years passing (an earlier first-generation product, Fluosol-DA 20% [Green Cross Corp, Osaka, Japan], had received FDA approval in 1989 for use in balloon angioplasty but was withdrawn in 1994 due to its side effect profile) [1]. The second approach toward development of a blood substitute involves hemoglobin-based oxygen carriers (HBOCs), using human, bovine, and recombinant Hgb sources [2, 3]. However, once again, successful development of a clinically effective product has been elusive because of the challenges and toxicities that must be successfully overcome. To begin with, cell-free Hgb is quite toxic to kidneys and other organs, and it leads to vasoconstriction and hypertension through exposure to endothelium and depletion of nitric oxide (a natural protector of vascular endothelium) as well as through development of toxic free radicals (the full range of cell-free Hgb toxicity is far too complex to discuss here, and so, the reader is referred to recommended reading below). Furthermore, cell-free Hgb has a short half-life and binds oxygen tightly (i.e., it exhibits a left shift in the Hgb–oxygen dissociation curve). To overcome these problems, manufacturers of HBOCs have used encapsulation, polymerization, and pyridoxilation, though even so, such products (which include Hemopure® [Biopure Corp, Cambridge, MA], Hemolink™ [Hemosol Inc., Toronto, Canada], PolyHeme® [Northfield Laboratories, Evanston, IL], and HemAssist®, Baxter International Inc., Deerfield, IL]) have failed clinical trials [2, 4]. More recently, a promising new HBOC has been introduced; this new product, known as PEGylated bovine carboxyhemoglobin (SANGUINATE™, Prolong Pharmaceuticals, LLC, South Plainfield, NJ), has been found to be beneficial in optimizing the delivery of oxygen, preventing vasoconstriction, and reducing inflammation [5]. The agent, which has now achieved orphan drug status by the FDA for sickle cell anemia (a designation that incentivizes manufacturers to develop drugs or biological agents for treatment of rare conditions), may be useful for treatment of vaso-occlusive crisis, stroke, and acute chest syndrome in sickle cell disease [6]. It may also prove to be useful in the treatment of other patients with severe life-threatening anemia for whom blood is not an option, though further studies are necessary for final FDA approval. Finally, it is important to understand that even when it comes to blood substitutes, not all "bloodless" patients will choose to accept such products since, depending on the product, it may be derived from a blood source (i.e., Hgb-derived).

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